24745

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 14/435, 14/705, A61K 38/03, 38/08, 38/17

A1

(11) International Publication Number: WO 00/52044

(43) International Publication Date: 8 September 2000 (08.09.00)

(21) International Application Number: PCT/US00/05612

(22) International Filing Date: 2 March 2000 (02.03.00)

(30) Priority Data:
09/261,416 3 March 1999 (03.03.99) US

(71) Applicant: THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS [US/US]; 2404 North University Avenue, Little Rock, AR 72207-3608 (US).

(72) Inventors: O'BRIEN, Timothy, J.; 2610 North Pierce, Little Rock, AR 72207 (US). UNDERWOOD, Lowell, J.; Apartment K, 121 N. Jackson Street, Little Rock, AR 72205 (US).

(74) Agent: ADLER, Benjamin, A.; McGregor & Adler, 8011 Candle Lane, Houston, TX 77071 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: TRANSMEMBRANE SERINE PROTEASE OVEREXPRESSED IN OVARIAN CARCINOMA AND USES THEREOF

(57) Abstract

The present invention provides a TADG-12 protein and a DNA fragment encoding such protein. Also provided is a vector/host cell capable of expressing the DNA. The present invention further provided various methods of early detection of associated ovarian and other malignancies, and of interactive therapies for cancer treatment by utilizing the DNA and/or protein disclosed herein.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

TRANSMEMBRANE SERINE PROTEASE OVEREXPRESSED IN OVARIAN CARCINOMA AND USES THEREOF

BACKGROUND OF THE INVENTION

Cross-Reference to Related Application

This application is a continuation-in-part patent application and claims the benefit of priority under 35 USC §120 of USSN 09/261,416, filed March 3, 1999.

Field of the Invention

The present invention relates generally to the fields of cellular biology and diagnosis of neoplastic disease. More specifically, the present invention relates to a transmembrane serine protease termed Tumor Associated Differentially-Expressed Gene-12 (TADG-12), which is overexpressed in ovarian carcinoma.

Description of the Related Art

Tumor cells rely on the expression of a concert of proteases to be released from their primary sites and move to distant sites to inflict lethality. This metastatic nature is the result of an aberrant expression pattern of proteases by tumor cells and also by stromal cells surrounding the tumors [1-3]. For most tumors to become metastatic, they must degrade their surrounding extracellular matrix components, degrade basement

membranes to gain access to the bloodstream or lymph system, and repeat this process in reverse fashion to settle in a secondary host site [3-6]. All of these processes rely upon what now appears to be a synchronized protease cascade. In addition, tumor cells use the power of proteases to activate growth and angiogenic factors that allow the tumor to grow progressively [1]. Therefore, much research has been aimed at the identification of tumor-associated proteases and the inhibition of these enzymes for therapeutic means. More importantly, the secreted nature and/or high level expression of many of these proteases allows for their detection at aberrant levels in patient serum, e.g. the prostate-specific antigen (PSA), which allows for early diagnosis of prostate cancer [7].

Proteases have been associated directly with tumor growth, shedding of tumor cells and invasion of target organs. Individual classes of proteases are involved in, but not limited to (1) the digestion of stroma surrounding the initial tumor area, (2) the digestion of the cellular adhesion molecules to allow dissociation of tumor cells; and (3) the invasion of the basement membrane for metastatic growth and the activation of both tumor growth factors and angiogenic factors.

For many forms of cancer, diagnosis and treatment has improved dramatically in the last 10 years. However, the five year survival rate for ovarian cancer remains below 50% due in large part to the vague symptoms which allow for progression of the disease to an advanced stage prior to diagnosis [8]. Although the exploitation of the CA125 antigen has been useful as a marker for monitoring recurrence of ovarian cancer, it has not proven to be an ideal marker for early diagnosis. Therefore, new markers

that may be secreted or released from cells and which are highly expressed by ovarian tumors could provide a useful tool for the early diagnosis and for therapeutic intervention in patients with ovarian carcinoma.

The prior art is deficient in the lack of the complete identification of the proteases overexpressed in carcinoma, therefore, deficient in the lack of a tumor marker useful as an indicator of early disease, particularly for ovarian cancers. Specifically, TADG-12, a transmembrane serine protease, has not been previously identified in either nucleic acid or protein form. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

The present invention discloses TADG-12, a new member of the Tumor Associated Differentially-Expressed Gene (TADG) family, and a variant splicing form of TADG-12 (TADG-12V) that could lead to a truncated protein product. TADG-12 is a transmembrane serine protease overexpressed in ovarian carcinoma. The entire cDNA of TADG-12 has been identified (SEQ ID No. 1). This sequence encodes a putative protein of 454 amino acids (SEQ ID No. 2) which includes a potential transmembrane domain, an LDL receptor like domain, a scavenger receptor cysteine rich domain, and a serine protease domain. These features imply that TADG-12 is expressed at the cell surface, and it may be used as a molecular target for therapy or a diagnostic marker.

In one embodiment of the present invention, there is provided a DNA fragment encoding a TADG-12 protein selected from the group consisting of: (a) an isolated DNA fragment which encodes a TADG-12 protein; (b) an isolated DNA fragment which hybridizes to isolated DNA fragment of (a) above and which encodes a TADG-12 protein; and (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Specifically, the DNA fragment has a sequence shown in SEQ ID No. 1 or SEQ ID No. 3.

In another embodiment of the present invention, there is provided a vector/host cell capable of expressing the DNA of the present invention.

In yet another embodiment of the present invention, there is provided an isolated and purified TADG-12 protein encoded by DNA selected from the group consisting of: (a) isolated DNA which encodes a TADG-12 protein; (b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a TADG-12 protein; and (c) isolated DNA differing from the isolated DNAs of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

In still yet another embodiment of the present invention, there is provided a method for detecting expression of a TADG-12 protein, comprising the steps of: (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

The present invention further provides methods for diagnosing a cancer or other malignant hyperplasia by detecting the TADG-12 protein or mRNA disclosed herein.

In still another embodiment of the present invention, there is provided a method of inhibiting expression of endogenous TADG-12 mRNA in a cell by introducing a vector into the cell, wherein the vector comprises a DNA fragment of TADG-12 in opposite orientation operably linked to elements necessary for expression.

In still yet another embodiment of the present invention, there is provided a method of inhibiting expression of a TADG-12 protein in a cell by introducing an antibody directed against a TADG-12 protein or fragment thereof.

In still yet another embodiment of the present invention, there is provided a method of targeted therapy by administering a compound having a targeting moiety specific for a TADG-12 protein and a therapeutic moiety. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

The present invention still further provides a method of vaccinating an individual against TADG-12 by inoculating the individual with a TADG-12 protein or fragment thereof. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. The TADG-12 fragment includes the truncated form of TADG-12V peptide having a sequence shown in SEQ ID No. 8, and a 9-residue up to 12-residue fragment of TADG-12 protein.

In yet another embodiment of the present invention, there is provided an immunogenic composition, comprising an

immunogenic fragment of a TADG-12 protein and an appropriate adjuvant. The TADG-12 fragment includes the truncated form of TADG-12V peptide having a sequence shown in SEQ ID No. 8, and a 9-residue up to 12-residue fragment of TADG-12 protein.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figure 1A shows that the expected PCR product of approximately 180 bp and the unexpected PCR product of approximately 300 bp using the redundant serine protease primers were not amplified from normal ovary cDNA (Lane 1) but were found in abundance from ovarian tumor cDNA (Lane 2). The primer sequences for the PCR reactions are indicated by horizontal arrows. **Figure 1B** shows that TADG-12 was subcloned from the 180 bp band while the larger 300 bp band was designated TADG-

12V. The sequences were found to overlap for 180 bp (SEQ ID No. 5 for nucleotide sequence, SEQ ID No. 6 for deduced amino acid sequence) with the 300 bp TADG-12V (SEQ ID No. 7 for nucleotide sequence, SEQ ID No. 8 for deduced amino acid sequence) having an additional insert of 133 bases. This insertion (vertical arrow) leads to a frame shift, which causes the TADG-12V transcript to potentially produce a truncated form of TADG-12 with a variant amino acid sequence.

Figure 2 shows that Northern blot analysis for TADG-12 revealed three transcripts of 2.4, 1.6 and 0.7 kilobases. These transcripts were found at significant levels in ovarian tumors and cancer cell lines, but the transcripts were found only at low levels in normal ovary.

Figure 3 shows an RNA dot blot (CLONTECH) probed for TADG-12. The transcript was detectable (at background levels) in all 50 of the human tissues represented with the greatest abundance of transcript in the heart. Putamen, amygdala, kidney, liver, small intestine, skeletal muscle, and adrenal gland were also found to have intermediate levels of TADG-12 transcript.

Figure 4 shows the entire cDNA sequence for TADG-12 (SEQ ID No. 1) with its predicted open reading frame of 454 amino acids (SEQ ID No. 2). Within the nucleotide sequence, the Kozak's consensus sequence for the initiation of translation and the poly-adenylation signal are underlined. In the protein sequence, a potential transmembrane domain is boxed. The LDLR-A domain is underlined with a solid line. The SRCR domain is underlined with a broken line. The residues of the catalytic triad of the serine protease domain are circled, and the beginning of the

catalytic domain is marked with an arrow designated as a potential proteolytic cleavage site. The * represents the stop codon that terminates translation.

Figure 5A shows the 35 amino acid LDLR-A domain of TADG-12 (SEQ ID No. 13) aligned with other LDLR-A motifs from the serine protease TMPRSS2 (U75329, SEQ ID No. 14), the complement subunit C8 (P07358, SEQ ID No. 9), two LDLR-A domains of the glycoprotein GP300 (P98164, SEQ ID Nos. 11-12), and the serine protease matriptase (AF118224, SEQ ID No. 10). TADG-12 has its highest similarity with the other serine proteases for which it is 54% similar to TMPRSS2 and 53% similar to matriptase. The highly conserved cysteine residues are shown in bold type. **Figure 5B** shows the SRCR domain of TADG-12 (SEQ ID No. 17) aligned with other domain family members including the human macrophage scavenger receptor (P21757, SEQ ID No. 16), human enterokinase (P98073, SEQ ID No. 19), bovine enterokinase (P21758, SEQ ID No. 15), and the serine protease TMPRSS2 (SEQ ID No. 18). Again, TADG-12 shows its highest similarity within this region to the protease TMPRSS2 at 43%. **Figure 5C** shows the protease domain of TADG-12 (SEQ ID No. 23) in alignment with other human serine proteases including protease M (U62801, SEQ ID No. 20), trypsinogen I (P07477, SEQ ID No. 21), plasma kallikrein (P03952, SEQ ID No. 22), hepsin (P05981, SEQ ID No. 25), and TMPRSS2 (SEQ ID No. 24). Cons represents the consensus sequence for each alignment.

Figure 6 shows semi-quantitative PCR analysis that was performed for TADG-12 (upper panel) and TADG-12V (lower panel). The amplification of TADG-12 or TADG-12V was performed in parallel with PCR amplification of β-tubulin product

as an internal control. The TADG-12 transcript was found to be overexpressed in 41 of 55 carcinomas. The TADG-12V transcript was found to be overexpressed in 8 of 22 carcinomas examined. Note that the samples in the upper panel are not necessarily the same as the samples in the lower panel.

Figure 7 shows immunohistochemical staining of normal ovary and ovarian tumors which were performed using a polyclonal rabbit antibody developed to a TADG-12 specific peptide. No significant staining was detected in normal ovary (**Figure 7A**). Strong positive staining was observed in 22 of 29 carcinomas examined. **Figures 7B** and **7C** represent a serous and mucinous carcinoma, respectively. Both show diffuse staining throughout the cytoplasm of tumor cells while stromal cells remain relatively unstained.

Figure 8 is a model to demonstrate the progression of TADG-12 within a cellular context. In normal circumstances, the TADG-12 transcript is appropriately spliced and the resulting protein is capable of being expressed at the cell surface where the protease may be cleaved to an active form. The role of the remaining ligand binding domains has not yet been determined, but one can envision their potential to bind other molecules for activation, internalization or both. The TADG-12V transcript, which occurs in some tumors, may be the result of mutation and/or poor mRNA processing may be capable of producing a truncated form of TADG-12 that does not have a functional protease domain. In addition, this truncated product may present a novel epitope at the surface of tumor cells.

DETAILED DESCRIPTION OF THE INVENTION

To examine the serine proteases expressed by ovarian cancers, a PCR based differential display technique was employed utilizing redundant PCR primers designed to the most highly conserved amino acids in these proteins [9]. As a result, a novel cell-surface, multi-domain serine protease, named Tumor Associated Differentially-expressed Gene-12 (TADG-12) was identified. TADG-12 appears to be overexpressed in many ovarian tumors. The extracellular nature of TADG-12 may render tumors susceptible to detection via a TADG-12 specific assay. In addition, a splicing variant of TADG-12, named TADG-12V, was detected at elevated levels in 35% of the tumors that were examined. TADG-12V encodes a truncated form of TADG-12 with an altered amino acid sequence that may be a unique tumor specific target for future therapeutic approaches.

The TADG-12 cDNA is 2413 base pairs long (SEQ ID No. 1) encoding a 454 amino acid protein (SEQ ID No. 2). A variant form, TADG-12V (SEQ ID No. 3), encodes a 294 amino acid protein (SEQ ID No. 4). The availability of the TADG-12 and/or TADG-12V gene opens the way for a number studies that can lead to various applications. For example, the TADG-12 and/or TADG-12V gene can be used as a diagnostic or therapeutic target in ovarian carcinoma and other carcinomas including breast, prostate, lung and colon.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis,

Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription and Translation" [B.D. Hames & S.J. Higgins eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

As used herein, the term "cDNA" shall refer to the DNA copy of the mRNA transcript of a gene.

As used herein, the term "derived amino acid sequence" shall mean the amino acid sequence determined by reading the triplet sequence of nucleotide bases in the cDNA.

As used herein the term "screening a library" shall refer to the process of using a labeled probe to check whether, under the appropriate conditions, there is a sequence complementary to the probe present in a particular DNA library. In addition, "screening a library" could be performed by PCR.

As used herein, the term "PCR" refers to the polymerase chain reaction that is the subject of U.S. Patent Nos. 4,683,195 and 4,683,202 to Mullis, as well as other improvements now known in the art.

The amino acid described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property of immunoglobulin-binding is retained by the polypeptide. NH_2 refers to the free amino group present at

the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J Biol. Chem.*, 243:3552-59 (1969), abbreviations for amino acid residues are known in the art.

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure herein according to the normal convention of giving only the sequence in

the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for

the binding of RNA polymerase. Eukaryotic promoters often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

A "signal sequence" can be included near the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

The term "oligonucleotide", as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which

is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementary with the sequence or hybridize therewith and thereby form the template for the synthesis of the extension product.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the

cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90% or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the

gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. In another example, coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others. A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

Proteins can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, β-glucuronidase, β-D-glucosidase, β-D-

galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090, 3,850,752, and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

A particular assay system developed and utilized in the art is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantitiy of both the label after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

An assay useful in the art is known as a "cis/trans" assay. Briefly, this assay employs two genetic constructs, one of which is typically a plasmid that continually expresses a particular receptor of interest when transfected into an appropriate cell line, and the second of which is a plasmid that expresses a reporter such as luciferase, under the control of a receptor/ligand complex. Thus, for example, if it is desired to evaluate a compound as a ligand for a particular receptor, one of the plasmids would be a construct that results in expression of the receptor in the chosen cell line, while the second plasmid would possess a promoter linked to the luciferase gene in which the response element to the particular receptor is inserted. If the compound under test is an agonist for the receptor, the ligand will complex with the receptor, and the resulting complex will bind the response element and initiate transcription of the luciferase gene. The resulting chemiluminescence is then measured photometrically, and dose response curves are obtained and compared to those of known

ligands. The foregoing protocol is described in detail in U.S. Patent No. 4,981,784.

As used herein, the term "host" is meant to include not only prokaryotes but also eukaryotes such as yeast, plant and animal cells. A recombinant DNA molecule or gene which encodes a human TADG-12 protein of the present invention can be used to transform a host using any of the techniques commonly known to those of ordinary skill in the art. Especially preferred is the use of a vector containing coding sequences for the gene which encodes a huma TADG-12 protein of the present invention for purposes of prokaryote transformation. Prokaryotic hosts may include *E. coli*, *S. tymphimurium*, *Serratia marcescens* and *Bacillus subtilis*. Eukaryotic hosts include yeasts such as *Pichia pastoris*, mammalian cells and insect cells.

In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted DNA fragment are used in connection with the host. The expression vector typically contains an origin of replication, promoter(s), terminator(s), as well as specific genes which are capable of providing phenotypic selection in transformed cells. The transformed hosts can be fermented and cultured according to means known in the art to achieve optimal cell growth.

The invention includes a substantially pure DNA encoding a TADG-12 protein, a strand of which DNA will hybridize at high stringency to a probe containing a sequence of at least 15 consecutive nucleotides of the sequence shown in SEQ ID No. 1 or SEQ ID No. 3. The protein encoded by the DNA of this invention may share at least 80% sequence identity (preferably 85%, more preferably 90%, and most preferably 95%) with the amino acids

listed in SEQ ID No. 2 or SEQ ID No. 4. More preferably, the DNA includes the coding sequence of the nucleotides of Figure 4 (SEQ ID No. 1), or a degenerate variant of such a sequence.

The probe to which the DNA of the invention hybridizes preferably consists of a sequence of at least 20 consecutive nucleotides, more preferably 40 nucleotides, even more preferably 50 nucleotides, and most preferably 100 nucleotides or more (up to 100%) of the coding sequence of the nucleotides listed in Figure 4 (SEQ ID No. 1) or the complement thereof. Such a probe is useful for detecting expression of TADG-12 in a human cell by a method including the steps of (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

This invention also includes a substantially pure DNA containing a sequence of at least 15 consecutive nucleotides (preferably 20, more preferably 30, even more preferably 50, and most preferably all) of the region from nucleotides 1 to 2413 of the nucleotides listed in SEQ ID No. 1, or of the region from nucleotides 1 to 2544 of the nucleotides listed in SEQ ID No. 3. The present invention also comprises antisense oligonucleotides directed against this novel DNA. Given the teachings of the present invention, a person having ordinary skill in this art would readily be able to develop antisense oligonucleotides directed against this DNA.

By "high stringency" is meant DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., wash conditions of 65°C at a salt concentration of approximately 0.1 x SSC, or the functional equivalent thereof.

For example, high stringency conditions may include hybridization at about 42°C in the presence of about 50% formamide; a first wash at about 65°C with about 2 x SSC containing 1% SDS; followed by a second wash at about 65°C with about 0.1 x SSC.

By "substantially pure DNA" is meant DNA that is not part of a milieu in which the DNA naturally occurs, by virtue of separation (partial or total purification) of some or all of the molecules of that milieu, or by virtue of alteration of sequences that flank the claimed DNA. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence, e.g., a fusion protein. Also included is a recombinant DNA which includes a portion of the nucleotides shown in SEQ ID No. 3 which encodes an alternative splice variant of TADG-12 (TADG-12V).

The DNA may have at least about 70% sequence identity to the coding sequence of the nucleotides listed in SEQ ID No. 1 or SEQ ID No. 3, preferably at least 75% (e.g. at least 80%); and most preferably at least 90%. The identity between two sequences is a direct function of the number of matching or identical positions. When a subunit position in both of the two sequences is occupied by the same monomeric subunit, e.g., if a given position is occupied by an adenine in each of two DNA molecules, then they are identical at that position. For example, if

7 positions in a sequence 10 nucleotides in length are identical to the corresponding positions in a second 10-nucleotide sequence, then the two sequences have 70% sequence identity. The length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 100 nucleotides. Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705).

The present invention comprises a vector comprising a DNA sequence which encodes a human TADG-12 protein and the vector is capable of replication in a host which comprises, in operable linkage: a) an origin of replication; b) a promoter; and c) a DNA sequence coding for said protein. Preferably, the vector of the present invention contains a portion of the DNA sequence shown in SEQ ID No. 1 or SEQ ID No. 3. A "vector" may be defined as a replicable nucleic acid construct, e.g., a plasmid or viral nucleic acid. Vectors may be used to amplify and/or express nucleic acid encoding a TADG-12 protein. An expression vector is a replicable construct in which a nucleic acid sequence encoding a polypeptide is operably linked to suitable control sequences capable of effecting expression of the polypeptide in a cell. The need for such control sequences will vary depending upon the cell selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter and/or enhancer, suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Methods which are well known to those skilled in the art can be used to

construct expression vectors containing appropriate transcriptional and translational control signals. See for example, the techniques described in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual* (2nd Ed.), Cold Spring Harbor Press, N.Y. A gene and its transcription control sequences are defined as being "operably linked" if the transcription control sequences effectively control the transcription of the gene. Vectors of the invention include, but are not limited to, plasmid vectors and viral vectors. Preferred viral vectors of the invention are those derived from retroviruses, adenovirus, adeno-associated virus, SV40 virus, or herpes viruses.

By a "substantially pure protein" is meant a protein which has been separated from at least some of those components which naturally accompany it. Typically, the protein is substantially pure when it is at least 60%, by weight, free from the proteins and other naturally-occurring organic molecules with which it is naturally associated in *vivo*. Preferably, the purity of the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight. A substantially pure TADG-12 protein may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding an TADG-12 polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography such as immunoaffinity chromatography using an antibody specific for TADG-12, polyacrylamide gel electrophoresis, or HPLC analysis. A protein is substantially free of naturally associated components when it is separated from at least some of those contaminants which accompany it in its natural state. Thus, a protein which is

chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be, by definition, substantially free from its naturally associated components. Accordingly, substantially pure proteins include eukaryotic proteins synthesized in *E. coli*, other prokaryotes, or any other organism in which they do not naturally occur.

In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the TADG-12 protein. As used herein, "fragment," as applied to a polypeptide, will ordinarily be at least 10 residues, more typically at least 20 residues, and preferably at least 30 (e.g., 50) residues in length, but less than the entire, intact sequence. Fragments of the TADG-12 protein can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally occurring or recombinant TADG-12 protein, by recombinant DNA techniques using an expression vector that encodes a defined fragment of TADG-12, or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of TADG-12 (e.g., binding to an antibody specific for TADG-12) can be assessed by methods described herein. Purified TADG-12 or antigenic fragments of TADG-12 can be used to generate new antibodies or to test existing antibodies (e.g., as positive controls in a diagnostic assay) by employing standard protocols known to those skilled in the art. Included in this invention are polyclonal antisera generated by using TADG-12 or a fragment of TADG-12 as the immunogen in, e.g., rabbits. Standard protocols for monoclonal and polyclonal antibody production known to those skilled in this art are employed. The monoclonal antibodies generated by this procedure can be screened for the ability to identify recombinant

TADG-12 cDNA clones, and to distinguish them from known cDNA clones.

Further included in this invention are TADG-12 proteins which are encoded at least in part by portions of SEQ ID No. 1 or SEQ ID No. 3, e.g., products of alternative mRNA splicing or alternative protein processing events, or in which a section of TADG-12 sequence has been deleted. The fragment, or the intact TADG-12 polypeptide, may be covalently linked to another polypeptide, e.g. which acts as a label, a ligand or a means to increase antigenicity.

The invention also includes a polyclonal or monoclonal antibody which specifically binds to TADG-12. The invention encompasses not only an intact monoclonal antibody, but also an immunologically-active antibody fragment, e.g., a Fab or $(Fab)_2$ fragment; an engineered single chain Fv molecule; or a chimeric molecule, e.g., an antibody which contains the binding specificity of one antibody, e.g., of murine origin, and the remaining portions of another antibody, e.g., of human origin.

In one embodiment, the antibody, or a fragment thereof, may be linked to a toxin or to a detectable label, e.g. a radioactive label, non-radioactive isotopic label, fluorescent label, chemiluminescent label, paramagnetic label, enzyme label, or colorimetric label. Examples of suitable toxins include diphtheria toxin, *Pseudomonas* exotoxin A, ricin, and cholera toxin. Examples of suitable enzyme labels include malate hydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease,

urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase, etc. Examples of suitable radioisotopic labels include ^{3}H, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, etc.

Paramagnetic isotopes for purposes of *in vivo* diagnosis can also be used according to the methods of this invention. There are numerous examples of elements that are useful in magnetic resonance imaging. For discussions on *in vivo* nuclear magnetic resonance imaging, see, for example, Schaefer et al., (1989) *JACC* 14, 472-480; Shreve et al., (1986) *Magn. Reson. Med.* 3, 336-340; Wolf, G. L., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 93-95; Wesbey et al., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 145-155; Runge et al., (1984) *Invest. Radiol.* 19, 408-415. Examples of suitable fluorescent labels include a fluorescein label, an isothiocyalate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an ophthaldehyde label, a fluorescamine label, etc. Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, an aequorin label, etc.

Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy et al., (1976) *Clin. Chim. Acta* 70, 1-31; and Schurs et al., (1977) *Clin. Chim. Acta* 81, 1-40. Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide

method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method. All of these methods are incorporated by reference herein.

Also within the invention is a method of detecting TADG-12 protein in a biological sample, which includes the steps of contacting the sample with the labeled antibody, e.g., radioactively tagged antibody specific for TADG-12, and determining whether the antibody binds to a component of the sample.

As described herein, the invention provides a number of diagnostic advantages and uses. For example, the TADG-12 protein disclosed in the present invention is useful in diagnosing cancer in different tissues since this protein is highly overexpressed in tumor cells. Antibodies (or antigen-binding fragments thereof) which bind to an epitope specific for TADG-12, are useful in a method of detecting TADG-12 protein in a biological sample for diagnosis of cancerous or neoplastic transformation. This method includes the steps of obtaining a biological sample (e.g., cells, blood, plasma, tissue, etc.) from a patient suspected of having cancer, contacting the sample with a labeled antibody (e.g., radioactively tagged antibody) specific for TADG-12, and detecting the TADG-12 protein using standard immunoassay techniques such as an ELISA. Antibody binding to the biological sample indicates that the sample contains a component which specifically binds to an epitope within TADG-12.

Likewise, a standard Northern blot assay can be used to ascertain the relative amounts of TADG-12 mRNA in a cell or tissue obtained from a patient suspected of having cancer, in accordance with conventional Northern hybridization techniques

known to those of ordinary skill in the art. This Northern assay uses a hybridization probe, e.g. radiolabelled TADG-12 cDNA, either containing the full-length, single stranded DNA having a sequence complementary to SEQ ID No. 1 or SEQ ID No. 3, or a fragment of that DNA sequence at least 20 (preferably at least 30, more preferably at least 50, and most preferably at least 100 consecutive nucleotides in length). The DNA hybridization probe can be labeled by any of the many different methods known to those skilled in this art.

Antibodies to the TADG-12 protein can be used in an immunoassay to detect increased levels of TADG-12 protein expression in tissues suspected of neoplastic transformation. These same uses can be achieved with Northern blot assays and analyses.

The present invention is directed to DNA fragment encoding a TADG-12 protein selected from the group consisting of: (a) an isolated DNA fragment which encodes a TADG-12 protein; (b) an isolated DNA fragment which hybridizes to isolated DNA fragment of (a) above and which encodes a TADG-12 protein; and (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Preferably, the DNA has the sequence shown in SEQ ID No. 1 or SEQ ID No. 3. More preferably, the DNA encodes a TADG-12 protein having the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

The present invention is also directed to a vector and/or a host cell capable of expressing the DNA of the present invention. Preferably, the vector contains DNA encoding a TADG-

12 protein having the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. Representative host cells include bacterial cells, yeast cells, mammalian cells and insect cells.

The present invention is also directed to an isolated and purified TADG-12 protein coded for by DNA selected from the group consisting of: (a) isolated DNA which encodes a TADG-12 protein; (b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a TADG-12 protein; and (c) isolated DNA differing from the isolated DNAs of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Preferably, the isolated and purified TADG-12 protein has the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

The present invention is also directed to a method of detecting expression of the TADG-12 protein described herein, comprising the steps of: (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

A number of potential applications are possible for the TADG-12 gene and gene product including the truncated product TADG-12V.

In one embodiment of the present invention, there is provided a method for diagnosing a cancer by detecting a TADG-12 protein in a biological sample, wherein the presence or absence of a TADG-12 protein indicates the presence or absence of a cancer. Preferably, the biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells. Still preferably, the detection of TADG-12 protein is by means selected

from the group consisting of Northern blot, Western blot, PCR, dot blot, ELIZA sandwich assay, radioimmunoassay, DNA array chips and flow cytometry. Such method is used for detecting an ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer and other cancers in which TADG-12 is overexpressed.

In another embodiment of the present invention, there is provided a method for detecting malignant hyperplasia by detecting a TADG-12 protein or TADG-12 mRNA in a biological sample. Further by comprising the TADG-12 protein or TADG-12 mRNA to reference information, a diagnosis or a treatment can be provided. Preferably, PCR amplification is used for detecting TADG-12 mRNA, wherein the primers utilized are selected from the group consisting of SEQ ID Nos. 28-31. Still preferably, detection of a TADG-12 protein is by immunoaffinity to an antibody directed against a TADG-12 protein.

In still another embodiment of the present invention, there is provided a method of inhibiting expression of endogenous TADG-12 mRNA in a cell by introducing a vector comprising a DNA fragment of TADG-12 in opposite orientation operably linked to elements necessary for expression. As a result, the vector produces TADG-12 antisense mRNA in the cell, which hybridizes to endogenous TADG-12 mRNA, thereby inhibiting expression of endogenous TADG-12 mRNA.

In still yet another embodiment of the present invention, there is provided a method of inhibiting expression of a TADG-12 protein by introducing an antibody directed against a TADG-12 protein or fragment thereof. As a result, the binding of the antibody to the TADG-12 protein or fragment thereof inhibits the expression of the TADG-12 protein.

TADG-12 gene products including the truncated form can be used for targeted therapy. Specifically, a compound having a targeting moiety specific for a TADG-12 protein and a therapeutic moiety is administered to an individual in need of such treatment. Preferably, the targeting moiety is selected from the group consisting of an antibody directed against a TADG-12 protein and a ligand or ligand binding domain that binds a TADG-12 protein. The TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. Still preferably, the therapeutic moiety is selected from the group consisting of a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant and a cytotoxic agent. Such method can be used for treating an individual having a disease selected from the group consisting of ovarian cancer, lung cancer, prostate cancer, colon cancer and other cancers in which TADG-12 is overexpressed.

In yet another embodiment of the present invention, there is provided a method of vaccinating, or producing an immune response in, an individual against TADG-12 by inoculating the individual with a TADG-12 protein or fragment thereof. Specifically, the TADG-12 protein or fragment thereof lacks TADG-12 activity, and the inoculation elicits an immune response in the individual, thereby vaccinating the individual against TADG-12. Preferably, the individual has a cancer, is suspected of having a cancer or is at risk of getting a cancer. Still preferably, TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4, while TADG-12 fragment has a sequence shown in SEQ ID No. 8, or is a 9-residue fragment up to a 20-residue fragment. Examples of 9-residue fragment are shown in SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

In still yet another embodiment of the present invention, there is provided an immunogenic composition, comprising an immunogenic fragment of a TADG-12 protein and an appropriate adjuvant. Preferably, the immunogenic fragment of the TADG-12 protein has a sequence shown in SEQ ID No. 8, or is a 9-residue fragment up to a 20-residue fragment. Examples of 9-residue fragment are shown in SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Tissue collection and storage

Upon patient hysterectomy, bilateral salpingo-oophorectomy, or surgical removal of neoplastic tissue, the specimen is retrieved and placed on ice. The specimen was then taken to the resident pathologist for isolation and identification of specific tissue samples. Finally, the sample was frozen in liquid nitrogen, logged into the laboratory record and stored at -80°C. Additional specimens were frequently obtained from the Cooperative Human Tissue Network (CHTN). These samples were prepared by the CHTN and shipped on dry ice. Upon arrival, these specimens were logged into the laboratory record and stored at -80°C.

EXAMPLE 2

mRNA Extraction and cDNA Synthesis

Sixty-nine ovarian tumors (4 benign tumors, 10 low malignant potential tumors and 55 carcinomas) and 10 normal

ovaries were obtained from surgical specimens and frozen in liquid nitrogen. The human ovarian carcinoma cell lines SW 626 and Caov 3, the human breast carcinoma cell lines MDA-MB-231 and MDA-MB-435S were purchased from the American Type Culture Collection (Rockville, MD). Cells were cultured to sub-confluency in Dulbecco's modified Eagle's medium, supplemented with 10% (v/v) fetal bovine serum and antibiotics.

Extraction of mRNA and cDNA synthesis were carried out by the methods described previously [14-16]. mRNA was isolated by using a RiboSep mRNA isolation kit (Becton Dickinson Labware). In this procedure, poly A+ mRNA was isolated directly from the tissue lysate using the affinity chromatography media oligo(dT) cellulose. cDNA was synthesized with 5.0 µg of mRNA by random hexamer priming using 1st strand cDNA synthesis kit (CLONTECH).

EXAMPLE 3

PCR with Redundant Primers and Cloning of TADG-12 cDNA

Redundant primers, forward 5'-TGGGTIGTIACIGCIGCICA(CT)TG -3' (SEQ ID No. 26) and reverse 5'-A(AG)IA(AG)IGCIATITCITTICC-3' (SEQ ID No. 27), for the consensus sequences of amino acids surrounding the catalytic triad for serine proteases were used to compare the PCR products from normal and carcinoma cDNAs. The appropriate bands were ligated into Promega T-vector plasmid and the ligation product was used to transform JM109 cells (Promega) grown on selection media. After selection of individual colonies, they were cultured and plasmid DNA was isolated by means of the Wizard miniprep DNA purification system (Promega). Nucleotide sequencing was

performed using PRISM Ready Reaction Dye Deoxy terminator cycle sequencing kit (Applied Biosystems). Applied Biosystems Model 373A DNA sequencing system was used for direct cDNA sequence determination.

The original TADG-12 subclone was randomly labeled and used as a probe to screen an ovarian tumor cDNA library by standard hybridization techniques [11,15]. The library was constructed in λZAP using mRNA isolated from the tumor cells of a stage III/grade III ovarian adenocarcinoma patient. Three overlapping clones were obtained which spanned 2315 nucleotides. The final 99 nucleotides encoding the most 3' sequence including the poly A tail was identified by homology with clones available in the GenBank EST database.

EXAMPLE 4

Quantitative PCR

The mRNA overexpression of TADG-12 was determined using a quantitative PCR. Quantitative PCR was performed according to the procedure as previously reported [16]. Oligonucleotide primers were used for: TADG-12, forward 5'-GAAACATGTCCTTGCTCTCG-3' (SEQ ID No. 28) and reverse 5'-ACTAACTTCCACAGCCTCCT-3' (SEQ ID No. 29); the variant TADG-12, forward 5'-TCCAGGTGGGTCTAGTTTCC-3' (SEQ ID No. 30), reverse 5'-CTCTTTGGCTTGTACTTGCT-3' (SEQ ID No. 31); β-tubulin, forward 5'- CGCATCAACGTGTACTACAA -3' (SEQ ID No. 32) and reverse 5'-TACGAGCTGGTGGACTGAGA -3' (SEQ ID No. 33). β-tubulin was utilized as an internal control. The PCR reaction mixture consists of cDNA derived from 50 ng of mRNA, 5 pmol of sense and antisense primers for both the TADG-12 gene and the β-tubulin

gene, 200 μmol of dNTPs, 5 μCi of α-^{32}PdCTP and 0.25 unit of Taq DNA polymerase with reaction buffer (Promega) in a final volume of 25 μl. The target sequences were amplified in parallel with the β-tubulin gene. Thirty cycles of PCR were carried out in a Thermal Cycler (Perkin-Elmer Cetus). Each cycle of PCR included 30 seconds of denaturation at 94%C, 30 seconds of annealing at 60%C and 30 seconds of extension at 72%C. The PCR products were separated on 2% agarose gels and the radioactivity of each PCR product was determined by using a Phospho Imager (Molecular Dynamics). The present study used the expression ratio (TADG-12/β-tubulin) as measured by phosphoimager to evaluate gene expression and defined the value at mean + 2SD of normal ovary as the cut-off value to determine overexpression. The student's *t* test was used for comparison of the mean values of normal ovary and tumors.

EXAMPLE 5

Sequencing of TADG-12/TADG-12V

Utilizing a plasmid specific primer near the cloning site, sequencing reactions were carried out using PRISM™ Ready Reaction Dye Deoxy™ terminators (Applied Biosystems cat# 401384) according to the manufacturer's instructions. Residual dye terminators were removed from the completed sequencing reaction using a Centri-sep™ spin column (Princeton Separation cat.# CS-901). An Applied Biosystems Model 373A DNA Sequencing System was available and was used for sequence analysis.

EXAMPLE 6

Antibody Production

Polyclonal rabbit antibodies were generated by immunization of white New Zealand rabbits with a poly-lysine linked multiple antigen peptide derived from the TADG-12 carboxy-terminal protein sequence NH_2-WIHEQMERDLKT-COOH (WIHEQMERDLKT, SEQ ID No. 34). This peptide is present in full length TADG-12, but not TADG-12V. Rabbits were immunized with approximately 100 µg of peptide emulsified in Ribi adjuvant. Subsequent boost immunizations were carried out at 3 and 6 weeks, and rabbit serum was isolated 10 days after the boost inoculations. Sera were tested by dot blot analysis to determine affinity for the TADG-12 specific peptide. Rabbit pre-immune serum was used as a negative control.

EXAMPLE 7

Northern Blot Analysis

10 µg of mRNA were loaded onto a 1% formaldehyde-agarose gel, electrophoresed and blotted on a Hybond-N+ nylon membrane (Amersham). ^{32}P-labeled cDNA probes were made by Prime-a-Gene Labeling System (Promega). The PCR products amplified by the same primers as above were used for probes. The blots were prehybridized for 30 min and hybridized for 60 min at 68%C with ^{32}P-labeled cDNA probe in ExpressHyb Hybridization Solution (CLONTECH). Control hybridization to determine relative gel loading was performed with the β-tubulin probe.

Normal human tissues; spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocyte, and normal human fetal tissues; brain, lung, liver and kidney (Human Multiple Tissue Northern Blot; CLONTECH) were also examined by same hybridization procedure.

EXAMPLE 8

Immunohistochemistry

Immunohistochemical staining was performed using a Vectastain Elite ABC Kit (Vector). Formalin fixed and paraffin embedded specimens were routinely deparaffinized and processed using microwave heat treatment in 0.01 M sodium citrate buffer (pH 6.0). The specimens were incubated with normal goat serum in a moist chamber for 30 minutes. TADG-12 peptide antibody was allowed to incubate with the specimens in a moisture chamber for 1 hour. Excess antibody was washed away with phosphate buffered saline. After incubation with biotinylated anti-rabbit IgG for 30 minutes, the sections were then incubated with ABC reagent (Vector) for 30 minutes. The final products were visualized using the AEC substrate system (DAKO) and sections were counterstained with hematoxylin before mounting. Negative controls were performed by using normal serum instead of the primary antibody.

EXAMPLE 9

Isolation of Catalytic Domain Subclones of TADG-12 and TADG-12 Variant

To identify serine proteases that are expressed in ovarian tumors, redundant PCR primers designed to the conserved

regions of the catalytic triad of these enzymes were employed. A sense primer designed to the region surrounding the conserved histidine and an anti-sense primer designed to the region surrounding the conserved aspartate were used in PCR reactions with either normal ovary or ovarian tumor cDNA as template. In the reaction with ovarian tumor cDNA, a strong product band of the expected size of approximately 180 bp was observed as well as an unexpected PCR product of approximately 300 bp which showed strong expression in some ovarian tumor cDNA's (Figure 1A). Both of these PCR products were subcloned and sequenced. The sequence of the subclones from the 180bp band (SEQ ID No. 5) was found to be homologous to the sequence identified in the larger, unexpected band (SEQ ID No. 7) except that the larger band had an additional insert of 133 nucleotides (Figure 1B). The smaller product of the appropriate size encoded for a protein sequence (SEQ ID No. 6) homologous to other known proteases while the sequence with the insertion (SEQ ID No. 8) encoded for a frame shift from the serine protease catalytic domain and a subsequent premature translational stop codon. TADG-12 variants from four individual tumors were also subcloned and sequenced. It was found that the sequence and insert to be identical. The genomic sequences for these cDNA derived clones were amplified by PCR, examined and found to contain potential AG/GT splice sites that would allow for the variant transcript production.

EXAMPLE 10

Northern Blot Analysis of TADG-12 Expression

To examine transcript size and tissue distribution, the catalytic domain subclone was randomly labeled and used to

probe Northern blots representing normal ovarian tissue, ovarian tumors and the cancer cell lines SW626, CAOV3, HeLa, MD-MBA-435S and MD-MBA-231 (Figure 2). Three transcripts of 2.4, 1.6 and 0.7 kilobases were observed. In blots of normal and ovary tumor the smallest transcript size 0.7 kb was lowly expressed in normal ovary while all transcripts (2.4, 1.6 and 0.7 kb) were abundantly present in serous carcinoma. In addition, Northern blots representing the normal human tissues spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocyte, and normal human fetal tissues of brain, lung, liver and kidney were examined. The same three transcripts were found to be expressed weakly in all of these tissues (data not shown). A human β-tubulin specific probe was utilized as a control for relative sample loading. In addition, an RNA dot blot was probed representing 50 human tissues and determined that this clone is weakly expressed in all tissues represented (Figure 3). It was found most prominently in heart, with intermediate levels in putamen, amygdala, kidney, liver, small intestine, skeletal muscle, and adrenal gland.

EXAMPLE 11

Sequencing and Characterization of TADG-12

An ovarian tumor cDNA library constructed in λZAP was screened by standard hybridization techniques using the catalytic domain subclone as a probe. Two clones that overlapped with the probe were identified and sequenced and found to represent 2316 nucleotides. The 97 nucleotides at the 3' end of the transcript including the poly-adenylation signal and the poly (A) tail were identified by homology with clones available in

GenBank's EST database. This brought the total size of the transcript to 2413 bases (SEQ ID No. 1, Figure 4). Subsequent screening of GenBank's Genomic Database revealed that TADG-12 is homologous to a cosmid from chromosome 17. This cosmid has the accession number AC015555.

The identified cDNA includes an open reading frame that would produce a predicted protein of 454 amino acids (SEQ ID No. 2), named Tumor Associated Differentially-Expressed Gene 12 (TADG-12). The sequence has been submitted to the GenBank database and granted the accession # AF201380. Using homology alignment programs, this protein contains several domains including an amino-terminal cytoplasmic domain, a potential Type II transmembrane domain followed by a low-density lipoprotein receptor-like class A domain (LDLR-A), a scavenger receptor cysteine rich domain (SRCR), and an extracellular serine protease domain.

As predicted by the ™Pred program, TADG-12 contains a highly hydrophobic stretch of amino acids that could serve as a potential transmembrane domain, which would retain the amino terminus of the protein within the cytoplasm and expose the ligand binding domains and protease domain to the extracellular space. This general structure is consistent with other known transmembrane proteases including hepsin [17], and TMPRSS2 [18], and TADG-12 is particularly similar in structure to the TMPRSS2 protease.

The LDLR-A domain of TADG-12 is represented by the sequence from amino acid 74 to 108 (SEQ ID No. 13). The LDLR-A domain was originally identified within the LDL Receptor [19] as a series of repeated sequences of approximately 40 amino acids,

which contained 6 invariant cysteine residues and highly conserved aspartate and glutamate residues. Since that initial identification, a host of other genes have been identified which contain motifs homologous to this domain [20]. Several proteases have been identified which contain LDLR-A motifs including matriptase, TMPRSS2 and several complement components. A comparison of TADG-12 with other known LDLR-A domains is shown in Figure 5A. The similarity of these sequences range from 44 to 54% of similar or identical amino acids.

In addition to the LDLR-A domain, TADG-12 contains another extracellular ligand binding domain with homology to the group A SRCR family. This family of protein domains typically is defined by the conservation of 6 cysteine resides within a sequence of approximately 100 amino acids [23]. The SRCR domain of TADG-12 is encoded by amino acids 109 to 206 (SEQ ID No. 17), and this domain was aligned with other SRCR domains and found to have between 36 and 43% similarity (Figure 5B). However, TADG-12 only has 4 of the 6 conserved cysteine residues. This is similar to the SRCR domain found in the protease TMPRSS2.

The TADG-12 protein also includes a serine protease domain of the trypsin family of proteases. An alignment of the catalytic domain of TADG-12 with other known proteases is shown in Figure 5C. The similarity among these sequence ranges from 48 to 55%, and TADG-12 is most similar to the serine protease TMPRSS2 which also contains a transmembrane domain, LDLR-A domain and an SRCR domain. There is a conserved amino acid motif (RIVGG) downstream from the SRCR domain that is a potential cleavage/activation site common to many serine

proteases of this family [25]. This suggests that TADG-12 is trafficked to the cell surface where the ligand binding domains are capable of interacting with extracellular molecules and the protease domain is potentially activated. TADG-12 also contains conserved cysteine residues (amino acids 208 and 243) which in other proteases form a disulfide bond capable of linking the activated protease to the other extracellular domains.

EXAMPLE 12

Quantitative PCR Characterization of the Alternative Transcript

The original TADG-12 subclone was identified as highly expressed in the initial redundant-primer PCR experiment. The TADG-12 variant form (TADG-12V) with the insertion of 133 bp was also easily detected in the initial experiment. To identify the frequency of this expression and whether or not the expression level between normal ovary and ovarian tumors was different, a previously authenticated semi-quantitative PCR techniquc was employed [16]. The PCR analysis co-amplified a product for β-tubulin with either a product specific to TADG-12 or TADG-12V in the presence of a radiolabelled nucleotide. The products were separated by agarose gel electrophoresis and a phosphoimager was used to quantitate the relative abundance of each PCR product. Examples of these PCR amplification products are shown for both TADG-12 and TADG-12V in Figure 6. Normal expression was defined as the mean ratio of TADG-12 (or TADG-12V) to β-tubulin +/- 2SD as examined in normal ovarian samples. For tumor samples, overexpression was defined as >2SD from the normal TADG-12/β-tubulin or TADG-12V/β-tubulin ratio. The results are summarized in Table 1 and Table 2. TADG-12 was

found to be overexpressed in 41 of 55 carcinomas examined while the variant form was present at aberrantly high levels in 8 of 22 carcinomas. As determined by the student's t test, these differences were statistically significant ($p < 0.05$).

TABLE 1

Frequency of Overexpression of TADG-12 in Ovarian Carcinoma

Histology Type	TADG-12 (%)
Normal	0/16 (0%)
LMP-Serous	3/6 (50%)
LMP-Mucinous	0/4 (0%)
Serous Carcinoma	23/29 (79%)
Mucinous Carcinoma	7/12 (58%)
Endometrioid Carcinoma	8/8 (100%)
Clear Cell Carcinoma	3/6 (50%)
Benign Tumors	3/4 (75%)

Overexpression =more than two standard deviations above the mean for normal ovary

LMP = low malignant potential tumor

TABLE 2

Frequency of Overexpression of TADG-12V in Ovarian Carcinoma

Histology Type	TADG-12V (%)
Normal	0/10 (0%)
LMP-Serous	0/5 (0%)
LMP-Mucinous	0/3 (0%)
Serous Carcinoma	4/14 (29%)
Mucinous Carcinoma	3/5 (60%)
Endometrioid Carcinoma	1/3 (33%)
Clear Cell Carcinoma	N/D

Overexpression =more than two standard deviations above the mean for normal ovary; LMP = low malignant potential tumor

EXAMPLE 13

Immunohistochemical Analysis of TADG-12 in Ovarian Tumor Cells

In order to examine the TADG-12 protein, polyclonal rabbit anti-sera to a peptide located in the carboxy-terminal amino acid sequence was developed. These antibodies were used to examine the expression level of the TADG-12 protein and its localization within normal ovary and ovarian tumor cells by immuno-localization. No staining was observed in normal ovarian tissues (Figure 7A) while significant staining was observed in 22 of 29 tumors studied. Representative tumor samples are shown in Figures 7B and 7C. It should be noted that TADG-12 is found in a diffuse pattern throughout the cytoplasm indicative of a protein in a trafficking pathway. TADG-12 is also found at the cell surface in these tumor samples as expected. It should be noted that the

antibody developed and used for immunohistochemical analysis would not detect the TADG-12V truncated protein.

The results of the immunohistochemical staining are summarized in Table 3. 22 of 29 ovarian tumors showed positive staining of TADG-12, whereas normal ovarian surface epithelium showed no expression of the TADG-12 antigen. 8 of 10 serous adenocarcinomas, 8 of 8 mucinous adenocarcinomas, 1 of 2 clear cell carcinomas, and 4 of 6 endometroid carcinomas showed positive staining.

TABLE 3

Case	Stage	Histology	Grade	LN*	TADG12	Prognosis
1		Normal ovary			0 -	
2		Normal ovary			0 -	
3		Normal ovary			0 -	
4		Mucinous B		ND	0 -	Alive
5		Mucinous B		ND	1+	Alive
6	1 a	Serous LMP	G1	ND	1+	Alive
7	1 a	Mucinous LMP	G1	ND	1+	Alive
8	1 a	Mucinous CA	G1	ND	1+	Alive
9	1 a	Mucinous CA	G2	ND	1+	Alive
10	1 a	Endometrioid CA	G1	ND	0 -	Alive
11	1 c	Serous CA	G1	N	1+	Alive
12	1 c	Mucinous CA	G1	N	1+	Alive
13	1 c	Mucinous CA	G1	N	2+	Alive
14	1 c	Clear cell CA	G2	N	0 -	Alive
15	1 c	Clear cell CA	G2	N	0 -	Alive
16	2 c	Serous CA	G3	N	2+	Alive
17	3 a	Mucinous CA	G2	N	2+	Alive

18	3b	Serous CA	G1	ND	1+	Alive
19	3c	Serous CA	G1	N	0-	Dead
20	3c	Serous CA	G3	P	1+	Alive
21	3c	Serous CA	G2	P	2+	Alive
22	3c	Serous CA	G1	P	2+	Unknown
23	3c	Serous CA	G3	ND	2+	Alive
24	3c	Serous CA	G2	N	0-	Dead
25	3c	Mucinous CA	G1	P	2+	Dead
26	3c	Mucinous CA	G2	ND	1+	Unknown
27	3c	Mucinous CA	G2	N	1+	Alive
28	3c	Endometrioid CA	G1	P	1+	Dead
29	3c	Endometrioid CA	G2	N	0-	Alive
30	3c	Endometrioid CA	G2	P	1+	Dead
31	3c	Endometrioid CA	G3	P	1+	Alive
32	3c	Clear Cell CA	G3	P	2+	Dead

LN*= Lymph Node: B = Benign; N = Negative; P = Positive;
ND = Not Done

EXAMPLE 14

Peptide Ranking

For vaccine or immune stimulation, individual 9-mers to 11-mers of the TADG-12 protein were examined to rank the binding of individual peptides to the top 8 haplotypes in the general population [Parker et al., (1994)]. The computer program used for this analysis can be found at <http://www-bimas.dcrt.nih.gov/molbio/hla_bind/>. Table 4 shows the peptide ranking based upon the predicted half-life of each peptide's binding to a particular HLA allele. A larger half-life indicates a

stronger association with that peptide and the particular HLA molecule. The TADG-12 peptides that strongly bind to an HLA allele are putative immunogens, and are used to innoculate an individual against TADG-12.

TABLE 4

TADG-12 peptide ranking

HLA Type & Ranking	Start	Peptide	Predicted Dissociation$_{1/2}$	SEQ ID No.
HLA A0201				
1	40	ILSLLPFEV	685.783	35
2	144	AQLGFPSYV	545.316	36
3	225	LLSQWPWQA	63.342	37
4	252	WIITAAHCV	43.992	38
5	356	VLNHAAVPL	36.316	39
6	176	LLPDDKVTA	34.627	40
7	13	FSFRSLFGL	31.661	41
8	151	YVSSDNLRV	27.995	42
9	436	RVTSFLDWI	21.502	43
10	234	SLQFQGYHL	21.362	44
11	181	KVTALHHSV	21.300	45
12	183	TALHHSVYV	19.658	46
13	411	RLWKLVGAT	18.494	47
14	60	LILALAIGL	18.476	48
15	227	SQWPWQASL	17.977	49
16	301	RLGNDIALM	11.426	50
17	307	ALMKLAGPL	10.275	51
18	262	DLYLPKSWT	9.837	52
19	416	LVGATSFGI	9.001	53
20	54	SLGIIALIL	8.759	54

HLA A0205				
1	218	IVGGNMSLL	47.600	55
2	60	LILALAIGL	35.700	48
3	35	AVAAQILSL	28.000	56
4	307	ALMKLAGPL	21.000	51
5	271	IQVGLVSLL	19.040	57
6	397	CQGDSGGPL	16.800	58
7	227	SQWPWQASL	16.800	49
8	270	TIQVGLVSL	14.000	59
9	56	GIIALILAL	14.000	60
10	110	RVGGQNAVL	14.000	61
11	181	KVTALHHSV	12.000	45
12	151	YVSSDNLRV	12.000	42
13	356	VLNHAAVPL	11.900	39
14	144	AQLGFPSYV	9.600	36
15	13	FSFRSLFGL	7.560	41
16	54	SLGIIALIL	7.000	54
17	234	SLQFQGYHL	7.000	44
18	217	RIVGGNMSL	7.000	62
19	411	RLWKLVGAT	6.000	47
20	252	WIITAAHCV	6.000	38
HLA A1				
1	130	CSDDWKGHY	37.500	63
2	8	AVEAPFSFR	9.000	64
3	328	NSEENFPDG	2.700	65
4	3	ENDPPAVEA	2.500	66
5	98	DCKDGEDEY	2.500	67
6	346	ATEDGGDAS	2.250	68
7	360	AAVPLISNK	2.000	69

8	153	SSDNLRVSS	1.500	70
9	182	VTALHHSVY	1.250	71
10	143	CAQLGFPSY	1.000	72
11	259	CVYDLYLPK	1.000	73
12	369	ICNHRDVYG	1.000	74
13	278	LLDNPAPSH	1.000	75
14	426	CAEVNKPGV	1.000	76
15	32	DADAVAAQI	1.000	77
16	406	VCQERRLWK	1.000	78
17	329	SEENFPDGK	0.900	79
18	303	GNDIALMKL	0.625	80
19	127	KTMCSDDWK	0.500	81
20	440	FLDWIHEQM	0.500	82
HLA A24				
1	433	VYTRVTSFL	280.000	83
2	263	LYLPKSWTI	90.000	84
3	169	EFVSIDHLL	42.000	85
4	217	RIVGGNMSL	12.000	62
5	296	KYKPKRLGN	12.000	86
6	16	RSLFGLDDL	12.000	87
7	267	KSWTIQVGL	11.200	88
8	81	RSSFKCIEL	8.800	89
9	375	VYGGIISPS	8.000	90
10	110	RVGGQNAVL	8.000	91
11	189	VYVREGCAS	7.500	92
12	60	LILALAIGL	7.200	48
13	165	QFREEFVSI	7.200	93
14	271	IQVGLVSLL	7.200	57
15	56	GIIALILAL	7.200	60

16	10	EAPFSFRSL	7.200	94
17	307	ALMKLAGPL	7.200	51
18	407	CQERRLWKL	6.600	95
19	356	VLNHAAVPL	6.000	39
20	381	SPSMLCAGY	6.000	96
HLA B7				
1	375	VYGGIISPS	200.000	97
2	381	SPSMLCAGY	80.000	98
3	362	VPLISNKIC	80.000	99
4	35	AVAAQILSL	60.000	56
5	373	RDVYGGIIS	40.000	100
6	307	ALMKLAGPL	36.000	51
7	283	APSHLVEKI	24.000	101
8	177	LPDDKVTAL	24.000	102
9	47	EVFSQSSSL	20.000	103
10	110	RVGGQNAVL	20.000	91
11	218	IVGGNMSLL	20.000	55
12	36	VAAQILSLL	12.000	104
13	255	TAAHCVYDL	12.000	105
14	10	EAPFSFRSL	12.000	94
15	138	YANVACAQL	12.000	106
16	195	CASGHVVTL	12.000	107
17	215	SSRIVGGNM	10.00	108
18	298	KPKRLGNDI	8.000	109
19	313	GPLTFNEMI	8.000	110
20	108	CVRVGGQNA	5.000	111
HLA B8				
1	294	HSKYKPKRL	80.000	112
2	373	RDVYGGIIS	16.000	100

3	177	LPDDKVTAL	4.800	102
4	265	LPKSWTIQV	2.400	113
5	88	ELITRCDGV	2.400	114
6	298	KPKRLGNDI	2.000	109
7	81	RSSFKCIEL	2.000	89
8	375	VYGGIISPS	2.000	97
9	79	RCRSSFKCI	2.000	115
10	10	EAPFSFRSL	1.600	94
11	215	SSRIVGGNM	1.000	108
12	36	VAAQILSLL	0.800	104
13	255	TAAHCVYDL	0.800	116
14	381	SPSMLCAGY	0.800	98
15	195	CASGHVVTL	0.800	107
16	362	VPLISNKIC	0.800	99
17	138	YANVACAQL	0.800	106
18	207	ACGHRRGYS	0.400	117
19	154	SDNLRVSSL	0.400	118
20	47	EVFSQSSSL	0.400	103
HLA B2702				
1	300	KRLGNDIAL	180.000	119
2	435	TRVTSFLDW	100.000	120
3	376	YGGIISPSM	100.000	121
4	410	RRLWKLVGA	60.000	122
5	210	HRRGYSSRI	60.000	123
6	227	SQWPWQASL	30.000	49
7	109	VRVGGQNAV	20.000	124
8	191	VREGCASGH	20.000	125
9	78	YRCRSSFKC	20.000	126
10	113	GQNAVLQVF	20.000	127

11	91	TRCDGVSDC	20.000	128
12	38	AQILSLLPF	20.000	129
13	211	RRGYSSRIV	18.000	130
14	216	SRIVGGNMS	10.000	131
15	118	LQVFTAASW	10.000	132
16	370	CNHRDVYGG	10.000	133
17	393	GVDSCQGDS	10.000	134
18	235	LQFQGYHLC	10.000	135
19	271	IQVGLVSLL	6.000	57
20	408	CQERRLWKL	6.000	95
HLA B4403				
1	427	AEVNKPGVY	90.000	136
2	162	LEGQFREEF	40.000	137
3	9	VEAPFSFRS	24.000	138
4	318	NEMIQPVCL	12.000	139
5	256	AAHCVYDLY	9.000	140
6	98	DCKDGEDEY	9.000	67
7	46	FEVFSQSSS	8.000	141
8	38	AQILSLLPF	7.500	129
9	64	LAIGLGIHF	7.500	142
10	192	REGCASGHV	6.000	143
11	330	EENFPDGKV	6.000	144
12	182	VTALHHSVY	6.000	145
13	408	QERRLWKLV	6.000	146
14	206	TACGHRRGY	4.500	147
15	5	DPPAVEAPF	4.500	148
16	261	YDLYLPKSW	4.500	149
17	33	ADAVAAQIL	4.500	150
18	168	EEFVSIDHL	4.000	151

19	304	NDIALMKLA	3.750	152
20	104	DEYRCVRVG	3.600	153

Conclusion

In this study, a serine protease was identified by means of a PCR based strategy. By Northern blot, the largest transcript for this gene is approximately 2.4 kb, and it is found to be expressed at high levels in ovarian tumors while found at minimal levels in all other tissues examined. The full-length cDNA encoding a novel multi-domain, cell-surface serine protease was cloned, named TADG-12. The 454 amino acid protein contains a cytoplasmic domain, a type II transmembrane domain, an LDLR-A domain, an SRCR domain and a serine protease domain. Using a semi-quantitative PCR analysis, it was shown that TADG-12 is overexpressed in a majority of tumors studied. Immunohistochemical staining corroborates that in some cases this protein is localized to the cell-surface of tumor cells and this suggests that TADG-12 has some extracellular proteolytic functions. Interestingly, TADG-12 also has a variant splicing form that is present in 35% of the tumors studied. This variant mRNA would lead to a truncated protein that may provide a unique peptide sequence on the surface of tumor cells.

This protein contains two extracellular domains which might confer unusual properties to this multidomain molecule. Although the precise role of LDLR-A function with regard to proteases remains unclear, this domain certainly has the capacity to bind calcium and other positively charged ligands [21,22]. This may play an important role in the regulation of the protease or

subsequent internalization of the molecule. The SRCR domain was originally identified within the macrophage scavenger receptor and functionally described to bind lipoproteins. Not only are SRCR domains capable of binding lipoproteins, but they may also bind to molecules as diverse as polynucleotides [23]. More recent studies have identified members of this domain family in proteins with functions that vary from proteases to cell adhesion molecules involved in maturation of the immune system [24]. In addition, TADG-12, like TMPRSS2 has only four of six cysteine residues conserved within its SRCR domain. This difference may allow for different structural features of these domains that confer unusual ligand binding properties. At this time, only the function of the CD6 encoded SRCR is well documented. In the case of CD6, the SRCR domain binds to the cell adhesion molecule ALCAM [23]. This mediation of cell adhesion is a useful starting point for future research on newly identified SRCR domains, however, the possibility of multiple functions for this domain can not be overlooked. SRCR domains are certainly capable of cell adhesion type interactions, but their capacity to bind other types of ligands should be considered.

At this time, the precise role of TADG-12 remains unclear. Substrates have not been identified for the protease domain, nor have ligands been identified for the extracellular LDLR-A and SRCR domains. Figure 8 presents a working model of TADG-12 with the information disclosed in the present invention. Two transcripts are produced which lead to the production of either TADG-12 or the truncated TADG-12V proteins. Either of these proteins is potentially targeted to the cell surface. TADG-12 is capable of becoming an activated serine protease while TADG-

12V is a truncated protein product that if at the cell surface may represent a tumor specific epitope.

The problem with treatment of ovarian cancer today remains the inability to diagnose the disease at an early stage. Identifying genes that are expressed early in the disease process such as proteases that are essential for tumor cell growth [26] is an important step toward improving treatment. With this knowledge, it may be possible to design assays to detect the highly expressed genes such as the TADG-12 protease described here or previously described proteases to diagnose these cancers at an earlier stage. Panels of markers may also provide prognostic information and could lead to therapeutic strategies for individual patients. Alternatively, inhibition of enzymes such as proteases may be an effective means for slowing progression of ovarian cancer and improving the quality of patient life. Other features of TADG-12 and TADG-12V must be considered important to future research too. The extracellular ligand binding domains are natural targets for drug delivery systems. The aberrant peptide associated with the TADG-12V protein may provide an excellent target drug delivery or for immune stimulation.

The following references were cited herein.

1. Duffy, M.J., Clin. Exp. Metastasis, 10: 145-155, 1992.
2. Monsky, W.L., et al., Cancer Res., 53: 3159-3164, 1993.
3. Powell, W.C., et al., Cancer Res., 53: 417-422, 1993.
4. Neurath, H. The Diversity of Proteolytic Enzymes. In: R.J. Beynon and J.S. Bond (eds.), pp. 1- 13, Proteolytic enzymes, Oxford: IRL Press, 1989.
5. Liotta, L.A., et al., Cell, 64: 327-336, 1991.

6. Tryggvason, K., et al., Biochem. Biophys. Acta., 907: 191-217, 1987.
7. McCormack, R.T., et al., Urology, 45:729-744, 1995.
8. Landis, S.H., et al., CA Cancer J. Clin., 48: 6-29, 1998.
9. Tanimoto, H., et al., Cancer Res., 57: 2884-2887, 1997.
10. Tanimoto, H., et al., Cancer, 86: 2074-2082, 1999.
11. Underwood, L.J., et al., Cancer Res., 59:4435-4439, 1999.
12. Tanimoto, et al., Increased Expression of Protease M in Ovarian Tumors. Tumor Biology, In Press, 2000.
13. Tanimoto, H., et al., Proc. Of the Amer. Assoc. for Canc. Research 39:648, 1998.
14. Tanimoto, H., et al., Tumor Biology, 20: 88-98, 1999.
15. Maniatis, T., Fritsch, E.F. & Sambrook, J. Molecular Cloning, p. 309-361 Cold Spring Harbor Laboratory, New York, 1982.
16. Shigemasa, K., et al., J. Soc. Gynecol. Invest., *4*:95-102, 1997.
17. Leytus, S.P., et al., Biochemistry, *27:* 1067-1074, 1988.
18. Paoloni-Giacobino, A., et al., Genomics, 44: 309-320, 1997.
19. Sudhof, T.C., et al., Science, 228: 815-822, 1985.
20. Daly, N., et al., Proc. Natl. Acad. Sci. USA 92: 6334-6338, 1995.
21. Mahley, R.W., Science 240: 622-630, 1988.
22. Van Driel, I.R., et al., J. Biol. Chem. 262: 17443-17449, 1987.
23. Freeman, M., et al., Proc. Natl. Acad. Sci. USA 87: 8810-8814, 1990.
24. Aruffo, A., et al., Immunology Today 18(10): 498-504, 1997.
25. Rawlings, N.D., and Barrett, A.J., Methods Enzymology 244: 19-61, 1994.
26. Torres-Rosado, A., et al., Proc. Natl. Acad. Sci. USA, *90:* 7181-7185, 1993.

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A DNA fragment encoding Tumor Associated Differentially-Expressed Gene-12 (TADG-12) protein selected from the group consisting of:

(a) an isolated DNA fragment which encodes a TADG-12 protein;

(b) an isolated DNA fragment which hybridizes to isolated DNA fragment of (a) above and which encodes a TADG-12 protein; and

(c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein.

2. The DNA fragment of claim 1, wherein said DNA fragment has the sequence selected from the group consisting of SEQ ID No. 1 and SEQ ID No. 3.

3. The DNA fragment of claim 1, wherein said TADG-12 protein has the amino acid sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

4. A vector comprising the DNA fragment of claim 1 and regulatory elements necessary for expression of the DNA in a cell.

5. The vector of claim 4, wherein said DNA fragment encodes a TADG-12 protein having the amino acid

sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

6. A host cell transfected with the vector of claim 4, said vector expressing a TADG-12 protein.

7. The host cell of claim 6, wherein said cell is selected from the group consisting of a bacterial cell, a mammalian cell, a plant cell and an insect cell.

8. The host cell of claim 7, wherein said bacterial cell is *E. coli.*

9. An antisense oligonucleotide directed against the DNA fragment of claim 1.

10. An isolated and purified TADG-12 protein coded for by DNA selected from the group consisting of:

(a) isolated DNA which encodes a TADG-12 protein;

(b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a TADG-12 protein; and

(c) isolated DNA differing from the isolated DNAs of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein.

11. The isolated and purified TADG-12 protein of claim 10, wherein said TADG-12 protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

12. A method for detecting expression of the TADG-12 protein of claim 10, comprising the steps of:

(a) contacting mRNA obtained from a cell with a labeled hybridization probe; and

(b) detecting hybridization of the probe with the mRNA.

13. An antibody directed against the TADG-12 protein of claim 10.

14. A method for diagnosing a cancer in an individual, comprising the steps of:

(a) obtaining a biological sample from said individual; and

(b) detecting a TADG-12 protein in said sample, wherein the presence of a TADG-12 protein in said sample is indicative of the presence of a cancer in said individual, wherein the absence of a TADG-12 protein in said sample is indicative of the absence of a cancer in said individual.

15. The method of claim 14, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

16. The method of claim 14, wherein said detection of a TADG-12 protein is by means selected from the group consisting of Northern blot, Western blot, PCR, dot blot, ELIZA

sandwich assay, radioimmunoassay, DNA array chips and flow cytometry.

17. The method of claim 14, wherein said cancer is selected from the group consisting of ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer and other cancers in which TADG-12 is overexpressed.

18. A method for detecting malignant hyperplasia in a biological sample, comprising the steps of:

(a) isolating mRNA from said sample; and

(b) detecting TADG-12 mRNA in said sample, wherein the presence of said TADG-12 mRNA in said sample is indicative of the presence of malignant hyperplasia, wherein the absence of said TADG-12 mRNA in said sample is indicative of the absence of malignant hyperplasia.

19. The method of claim 18, further comprising the step of comparing said TADG-12 mRNA to reference information, wherein said comparison provides a diagnosis of said malignant hyperplasia.

20. The method of claim 18, further comprising the step of comparing said TADG-12 mRNA to reference information, wherein said comparison determines a treatment of said malignant hyperplasia.

21. The method of claim 18, wherein said detection of TADG-12 mRNA is by PCR amplification.

22. The method of claim 21, wherein said PCR amplification uses primers selected from the group consisting of SEQ ID Nos. 28-31.

23. The method of claim 18, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

24. A method for detecting malignant hyperplasia in a biological sample, comprising the steps of:

(a) isolating protein from said sample; and

(b)detecting a TADG-12 protein in said sample, wherein the presence of a TADG-12 protein in said sample is indicative of the presence of malignant hyperplasia, wherein the absence of a TADG-12 protein in said sample is indicative of the absence of malignant hyperplasia.

25. The method of claim 24, further comprising the step of comparing said TADG-12 protein to reference information, wherein said comparison provides a diagnosis of said malignant hyperplasia.

26. The method of claim 24, further comprising the step of comparing said TADG-12 protein to reference information, wherein said comparison determines a treatment of said malignant hyperplasia.

27. The method of claim 24, wherein said detection is by immunoaffinity to an antibody, wherein said antibody is directed against a TADG-12 protein.

28. The method of claim 24, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

29. A method of inhibiting expression of endogenous TADG-12 mRNA in a cell, comprising the step of:

introducing a vector into a cell, wherein said vector comprises a DNA fragment of TADG-12 in opposite orientation operably linked to elements necessary for expression, wherein expression of said vector in said cell produces TADG-12 antisense mRNA, wherein said TADG-12 antisense mRNA hybridizes to endogenous TADG-12 mRNA, thereby inhibiting expression of endogenous TADG-12 mRNA in said cell.

30. A method of inhibiting expression of a TADG-12 protein in a cell, comprising the step of:

introducing an antibody into a cell, wherein said antibody is directed against a TADG-12 protein or fragment thereof, wherein binding of said antibody to said TADG-12 protein or fragment thereof inhibits expression of said TADG-12 protein.

31. A method of targeted therapy to an individual, comprising the step of:

administering a compound to an individual, wherein said compound has a targeting moiety and a therapeutic moiety, wherein said targeting moiety is specific for a TADG-12 protein.

32. The method of claim 31, wherein said targeting moiety is selected from the group consisting of an antibody directed against a TADG-12 protein and a ligand or ligand binding domain that binds a TADG-12 protein.

33. The method of claim 32, wherein said TADG-12 protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

34. The method of claim 31, wherein said therapeutic moiety is selected from the group consisting of a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant and a cytotoxic agent.

35. The method of claim 31, wherein said individual suffers from a disease selected from the group consisting of ovarian cancer, lung cancer, prostate cancer, colon cancer and other cancers in which TADG-12 is overexpressed.

36. A method of vaccinating an individual against TADG-12, comprising the step of inoculating the individual with a TADG-12 protein or fragment thereof, wherein said TADG-12 protein or fragment thereof lacks TADG-12 activity, wherein said inoculation with said TADG-12 protein or fragment thereof elicits

an immune response in said individual, thereby vaccinating said individual against TADG-12.

37. The method of claim 36, wherein said individual has a cancer, is suspected of having a cancer or is at risk of getting a cancer.

38. The method of claim 36, wherein said TADG-12 protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

39. The method of claim 36, wherein said TADG-12 fragment has a sequence shown in SEQ ID No. 8.

40. The method of claim 36, wherein said TADG-12 fragment is a 9-residue fragment selected from the group consisting of SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

41. An immunogenic composition, comprising an immunogenic fragment of a TADG-12 protein and an appropriate adjuvant.

42. The immunogenic composition of claim 41, wherein said immunogenic fragment of a TADG-12 protein has a sequence shown in SEQ ID No. 8.

43. The immunogenic composition of claim 41, wherein said immunogenic fragment of a TADG-12 protein is a 9-residue fragment selected from the group consisting of SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

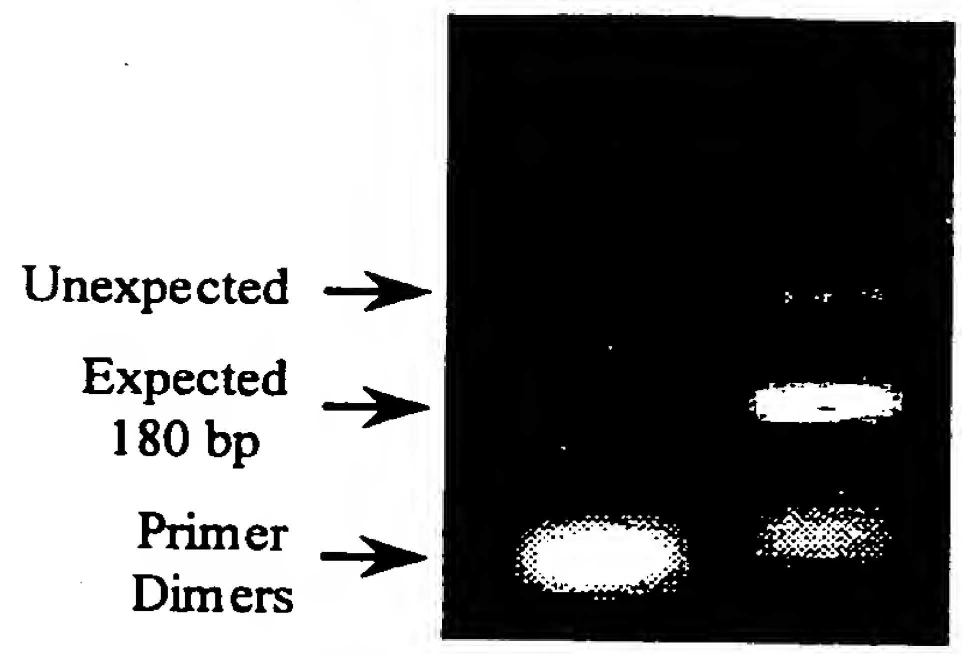


FIG. 1A

```
                         TADG12
  1  TGGGTGGTGACGGCGGCGCACTGTGTTTATGACTTGTACCTCCCCAAGTCATGGACCATC
     W  V  V  T  A  A  H  C  V  Y  D  L  Y  L  P  K  S  W  T  I

 61  CAGGTGGGTCTAGTTTCCCTGTTGGACAATCCAGCCCCATCCCACTTGGTGGAGAAGATT
     Q  V  G  L  V  S  L  L  D  N  P  A  P  S  H  L  V  E  K  I
                                                  (SEQ ID NO. 5)
121  GTCTACCACAGCAAGTACAAGCCAAAGAGGCTGGGCAACGACATCGCCCTCCTA
     V  Y  H  S  K  Y  K  P  K  R  L  G  N  D  I  A  L  L
                                                  (SEQ ID NO. 6)

                        TADG12-V
  1  GGGTGGTGACGGCGGCGCACTGTGTTTATGAGATTGTAGCTCCTAGAGAAAGGGCAGACA
        V  V  T  A  A  H  C  V  Y  E  I  V  A  P  R  E  R  A  D  R

 61  GAAGAGGAAGGAAGCTCCTGTGCTGGAGGAAACCCACAAAAATGAAAGGACCTAGACCTT
        R  G  R  K  L  L  C  W  R  K  P  T  K  M  K  G  P  R  P  S

121  CCCATAGCTAATTCCAGTGGACCATGTTATGGCAGATACAGGCTTGTACCTCCCCAAGTC
        H  S  *  (SEQ ID NO. 8)

181  ATGGACCATCCAGGTGGGTCTAGTTTCCCTGTTGGACAATCCAGCCCCATCCCACTTGGT
241  GGAGAAGATTGTCTACCACAGCAAGTACAAGCCAAAGAGGCTGGGCAACGACATCGCCCT
301  CCTAATCACTAGTGCGGCCGCCTGCAGG  (SEQ ID NO. 7)
```

FIG. 1B

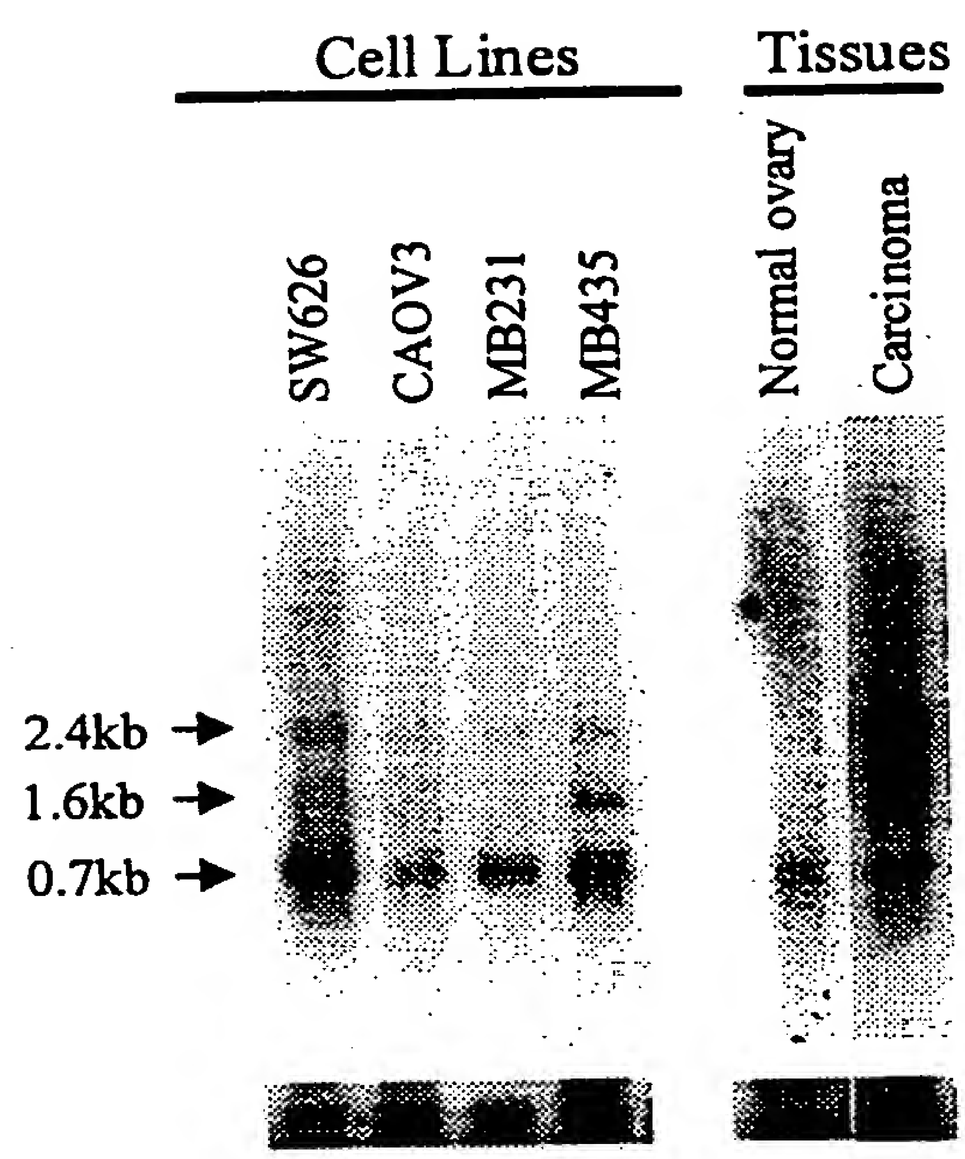
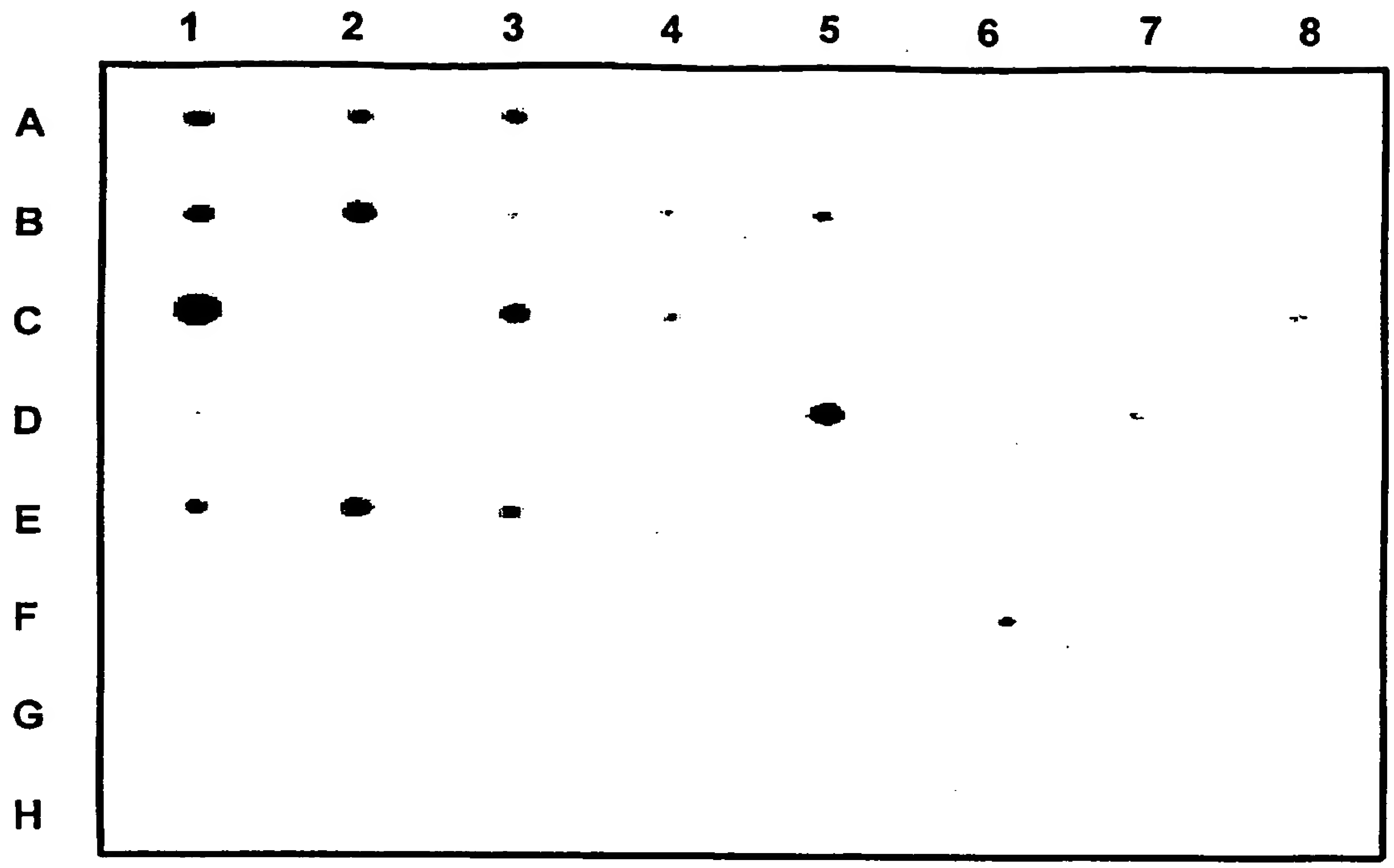

Cell Lines
Tissues
SW626
CAOV3
MB231
MB435
Normal ovary
Carcinoma
2.4kb
1.6kb
0.7kb

FIG. 2



A	whole brain	amydala	caudate nucleus	cere - bellum	cerebral cortex	frontal lobe	hippo - campus	medulla oblongata
B	occipital lobe	putamen	subst. nigra	temporal lobe	thalamus	sub - thalamic nucleus	spinal cord	
C	heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
D	testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
E	kidney	liver	small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
F	appendix	lung	trachea	placenta				
G	fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
H	yeast total RNA 100 ng	yeast tRNA 100 ng	E.coli rRNA 100 ng	E.coli DNA 100 ng	Poly r(A) 100 ng	human Cot1 DNA 100 ng	human DNA 100 ng	human DNA 500 ng

FIG. 3

```
   1 CGGGAAAGGGCTGTGTTTATGGGAAGCCAGTAACACTGTGGCCTACTATCTCTTCCGTGG
  61 TGCCATCTACATTTTTGGGACTCGGGAATTATGAGGTAGAGGTGGAGGCGGAGCCGGATG
 121 TCAGAGGTCCTGAAATAGTCACCATGGGGGAAAATGATCCGCCTGCTGTTGAAGCCCCCT
                             M  G  E  N  D  P P  A  V  E  A  P  F  13
 181 TCTCATTCCGATCGCTTTTTGGCCTTGATGATTTGAAAATAAGTCCTGTTGCACCAGATG
      S  F  R  S  L  F  G  L  DD  L  K  I  S  P  V  A  P  D  A  33
 241 CAGATGCTGTTGCTGCACAGATCCTGTCACTGCTGCCATTTGAAGTTTTTTCCCAATCAT
      D  A  V  A A  Q  I  L  S  L  L  P  F  E  V  F  S  Q  S  S  53
 301 CGTCATTGGGGATCATTGCATTGATATTAGCACTGGCCATTGGTCTGGGCATCCACTTCG
      S  L  G  I  I  A  L  I  L  A  L  A  I  G  L  G  I  H  F  D  73
 361 ACTGCTCAGGGAAGTACAGATGTCGCTCATCCTTTAAGTGTATCGAGCTGATAACTCGAT
      C  S  G  K  Y  R  C  R  S  S  F  K  C  I  E  L  I  T  R  C  93
 421 GTGACGGAGTCTCGGATTGCAAAGACGGGGAGGACGAGTACCGCTGTGTCCGGGTGGGTG
      D  G  V  S  D  C  K  D  G  E  D  E  Y  R  C  V  R  V  G  G 113
 481 GTCAGAATGCCGTGCTCCAGGTGTTCACAGCTGCTTCGTGGAAGACCATGTGCTCCGATG
      Q  N  A  V  L  Q  V  F  T  A  A  S  W  K  T  M  C  S  D  D 133
 541 ACTGGAAGGGTCACTACGCAAATGTTGCCTGTGCCCAACTGGGTTTCCCAAGCTATGTGA
      W  K  G  H  Y  A  N  V  A  C  A  Q  L  G  F  P  S  Y  V  S 153
 601 GTTCAGATAACCTCAGAGTGAGCTCGCTGGAGGGGCAGTTCCGGGAGGAGTTTGTGTCCA
      S  D  N  L  R  V  S  S  L  E  G  Q  F  R  E  E  F  V  S  I 173
 661 TCGATCACCTCTTGCCAGATGACAAGGTGACTGCATTACACCACTCAGTATATGTGAGGG
      D  H  L  L  P  D  D  K  V  T  A  L  H  H  S  V  Y  V  R  E 193
 721 AGGGATGTGCCTCTGGCCACGTGGTTACCTTGCAGTGCACAGCCTGTGGTCATAGAAGGG
      G  C  A  S  G  H  V  V  T  L  Q  C  T  A  C  G  H  R  R  G 213
 781 GCTACAGCTCACGCATCGTGGGTGGAAACATGTCCTTGCTCTCGCAGTGGCCCTGGCAGG
      Y  S  S  R  I  V  G  G  N  M  S  L  L  S  Q  W  P  W  Q  A 233
 841 CCAGCCTTCAGTTCCAGGGCTACCACCTGTGCGGGGGCTCTGTCATCACGCCCCTGTGGA
      S  L  Q  F  Q  G  Y  H  L  C  G  G  S  V  I  T  P  L  W  I 253
 901 TCATCACTGCTGCACACTGTGTTTATGACTTGTACCTCCCCAAGTCATGGACCATCCAGG
      I  T  A  A  H  C  V  Y  D  L  Y  L  P  K  S  W  T  I  Q  V 273
 961 TGGGTCTAGTTTCCCTGTTGGACAATCCAGCCCCATCCCACTTGGTGGAGAAGATTGTCT
      G  L  V  S  L  L  D  N  P  A  P  S  H  L  V  E  K  I  V  Y 293
1021 ACCACAGCAAGTACAAGCCAAAGAGGCTGGGCAATGACATCGCCCTTATGAAGCTGGCCG
      H  S  K  Y  K  P  K  R  L  G  N  D  I  A  L  M  K  L  A  G 313
1081 GGCCACTCACGTTCAATGAAATGATCCAGCCTGTGTGCCTGCCCAACTCTGAAGAGAACT
      P  L  T  F  N  E  M  I  Q  P  V  C  L  P  N  S  E  E  N  F 333
1141 TCCCCGATGGAAAAGTGTGCTGGACGTCAGGATGGGGGGCCACAGAGGATGGAGGTGACG
      P  D  G  K  V  C  W  T  S  G  W  G  A  T  E  D  G  G  D  A 353
1201 CCTCCCCTGTCCTGAACCACGCGGCCGTCCCTTTGATTTCCAACAAGATCTGCAACCACA
      S  P  V  L  N  H  A  A  V  P  L  I  S  N  K  I  C  N  H  R 373
1261 GGGACGTGTACGGTGGCATCATCTCCCCCTCCATGCTCTGCGCGGGCTACCTGACGGGTG
      D  V  Y  G  G  I  I  S  P  S  M  L  C  A  G  Y  L  T  G  G 393
1321 GCGTGGACAGCTGCCAGGGGGACAGCGGGGGGCCCCTGGTGTGTCAAGAGAGGAGGCTGT
      V  D  S  C  Q  G  D  S  G  G  P  L  V  C  Q  E  R  R  L  W 413
1381 GGAAGTTAGTGGGAGCGACCAGCTTTGGCATCGGCTGCGCAGAGGTGAACAAGCCTGGGG
      K  L  V  G  A  T  S  F  G  I  G  C  A  E  V  N  K  P  G  V 433
1441 TGTACACCCGTGTCACCTCCTTCCTGGACTGGATCCACGAGCAGATGGAGAGAGACCTAA
      Y  T  R  V  T  S  F  L  D  W  I  H  E  Q  M  E  R  D  L  K 453
1501 AAACCTGAAGAGGAAGGGGACAAGTAGCCACCTGAGTTCCTGAGGTGATGAAGACAGCCC
      T  *  (SEQ ID NO. 2)                                      454
1561 GATCCTCCCCTGGACTCCCGTGTAGGAACCTGCACACGAGCAGACACCCTTGGAGCTCTG
1621 AGTTCCGGCACCAGTAGCGGGCCCGAAAGAGGCACCCTTCCATCTGATTCCAGCACAACC
1681 TTCAAGCTGCTTTTTGTTTTTTGTTTTTTGAGGTGGAGTCTCGCTCTGTTGCCCAGGCT
1741 GGAGTGCAGTGGCGAAATACCCTGCTCACTGCAGCCTCCGCTTCCCTGGTTCAAGCGATT
1801 CTCTTGCCTCAGCTTCCCCAGTAGCTGGGACCACAGGTGCCCGCCACCACACCCAACTAA
1861 TTTTTGTATTTTTAGTAGAGACAGGGTTTCACCATGTTGGCCAGGCTGCTCTCAAACCCC
1921 TGACCTCAAATGATGTGCCTGCTTCAGCCTCCCACAGTGCTGGGATTACAGGCATGGGCC
1981 ACCACGCCTAGCCTCACGCTCCTTTCTGATCTTCACTAAGAACAAAAGAAGCAGCAACTT
2041 GCAAGGGCGGCCTTTCCCACTGGTCCATCTGGTTTTCTCTCCAGGGTCTTGCAAAATTCC
2101 TGACGAGATAAGCAGTTATGTGACCTCACGTGCAAAGCCACCAACAGCCACTCAGAAAAG
2161 ACGCACCAGCCCAGAAGTGCAGAACTGCAGTCACTGCACGTTTTCATCTTTAGGGACCAG
2221 AACCAAACCCACCCTTTCTACTTCCAAGACTTATTTTCACATGTGGGGAGGTTAATCTAG
2281 GAATGACTCGTTTAAGGCCTATTTTCATGATTTCTTTGTAGCATTTGGTGCTTGACGTAT
2341 TATTGTCCTTTGATTCCAAATAATATGTTTCCTTCCCTCAAAAAAAAAAAAAAAAAAAAA
2401 AAAAAAAAAAAAA (SEQ ID NO. 1)
```

FIG. 4

```
 Compc8  CEG..FVC AQTGRCVNRR LLCNGDNDCG DQSDEAN.C  (SEQ ID NO. 9)
   Matr  CPG.QFTC .RTGRCIRKE LRCDGWADCT DHSDELN.C  (SEQ ID NO. 10)
Gp300-1  CQQGYFKC QSEGQCIPSS WVCDQDQDCD DGSDERQDC  (SEQ ID NO. 11)
Gp300-2  CSSHQITC .SNGQCIPSE YRCDHVRDCP DGADE.NDC  (SEQ ID NO. 12)
 TADG12  CSGK.YRC RSSFKCIELI TRCDGVSDCK DGEDEYR.C  (SEQ ID NO. 13)
Tmprss2  CSNSGIEC DSSGTCINPS NWCDGVSHCP GGEDENR.C  (SEQ ID NO. 14)
   Cons  C        C          C          C    C    DE    C
```

FIG. 5A

```
BovEntk  VRLVGGSGPH EGRVEI.FHE GQWGTVCDDR WELRGGLVVC RSLGYKGVQS
  MacSR  VRLVGGSGPH EGRVEI.LHS GQWGTICDDR WEVRVGQVVC RSLGYPGVQA
 TADG12  VRVGG...QN AVLQVFTA.. ASWKTMCSDD WKGHYANVAC AQLGFP.SYV
Tmprss2  VRLYG...PN FILQMYSSQR KSWHPVCQDD WNENYGRAAC RDMGYKNNFY
HumEntk  VRFFNGTTNN NGLVRFRIQ. SIWHTACAEN WTTQISNDVC QLLGLGSG..
   Cons  VR                      W    C    W         C

BovEntk  VHKRAYFGKG TGPIWLNEVF CFGK..ESSI EECRIRQWGV R.ACSHDEDA
  MacSR  VHKAAHFGQG TGPIWLNEVF CFGR..ESSI EECKIRQWGT R.ACSHSEDA
 TADG12  SSDNLRVSSL EGQFREEFVS I.DHLLPDDK VTALHHSVYV REGCASGHVV
Tmprss2  SSQGIVDDSG STSFMKLNTS A.GNV...DI YKKLYHS... .DACSSKAVV
HumEntk  NSSKPIFSTD GGPFVKLNTA PDGHLILTPS QQ........ ...CLQDSLI
   Cons                                                C

BovEntk  GVTCT  (SEQ ID NO. 15)
  MacSR  GVTCT  (SEQ ID NO. 16)
 TADG12  TLQCT  (SEQ ID NO. 17)
Tmprss2  SLRCL  (SEQ ID NO. 18)
HumEntk  RLQC.  (SEQ ID NO. 19)
   Cons     C
```

FIG. 5B

```
ProM     LWVLTAAHCK ......KPNL QVFLGKHNLR QRESSQEQSS VVRAVIHPDY
Try1     QWVVSAGHCY ......KSRI QVRLGEHNIE VLEGNEQFIN AAKIIRHPQY
Kal      QWVLTAAHCF D.GLPLQDVW RIYSGILNLS DITKDTPFSQ IKEIIIHQNY
TADG12   LWIITAAHCV .YDLYLPKSW TIQVGLV..S LLDNPAPSHL VEKIVYHSKY
Tmprss2  EWIVTAAHCV EKPLNNPWHW TAFAGILRQS FMFYGA.GYQ VQKVISHPNY
Heps     DWVLTAAHCF PERNRVLSRW RVFAGAVAQA SPHGLQLG.. VQAVVYHGGY
Cons      W   A HC                G                       H  Y

ProM     ......DAAS HDQDIMLLRL ARPAKLSELI QPLPLERDCS ANT..TSCHI
Try1     ......DRKT LNNDIMLIKL SSRAVINARV STISLPTAPP ATG..TKCLI
Kal      ......KVSE GNHDIALIKL QAPLNYTEFQ KPICLPSKGD TSTIYTNCWV
TADG12   ......KPKR LGNDIALMKL AGPLTFNEMI QPVCLPNSEE NFPDGKVCWT
Tmprss2  ......DSKT KNNDIALMKL QKPLTFNDLV KPVCLPNPGM MLQPEQLCWI
Heps     LPFRDPNSEE NSNDIALVHL SSPLPLTEYI QPVCLPAAGQ ALVDGKICTV
Cons                    DI L  L                L               C

ProM     LGWGKTAD.. GDFPDTIQCA YIHLVSREEC EHA..YPGQI TQNMLCAGDE
Try1     SGWGNTASSG ADYPDELQCL DAPVLSQAKC EAS..YPGKI TSNMFCVGFL
Kal      TGWGFSKEK. GEIQNILQKV NIPLVTNEEC QKR.YQDYKI TQRMVCAGYK
TADG12   SGWGAT.EDG GDASPVLNHA AVPLISNKIC NHRDVYGGII SPSMLCAGYL
Tmprss2  SGWGAT.EEK GKTSEVLNAA KVLLIETQRC NSRYVYDNLI TPAMICAGFL
Heps     TGWGNT.QYY GQQAGVLQEA RVPIISNDVC NGADFYGNQI KPKMFCAGYP
Cons      GWG                                  C          I    M C G

ProM     KYGKDSCQGD SGGPLVC   (SEQ ID NO. 20)
Try1     EGGKDSCQGD SGGPVVC   (SEQ ID NO. 21)
Kal      EGGKDACKGD SGGPLVC   (SEQ ID NO. 22)
TADG12   TGGVDSCQGD SGGPLVC   (SEQ ID NO. 23)
Tmprss2  QGNVDSCQGD SGGPLVT   (SEQ ID NO. 24)
Heps     EGGIDACQGD SGGPFVC   (SEQ ID NO. 25)
Cons         D C GD SGGP V
```

FIG. 5C

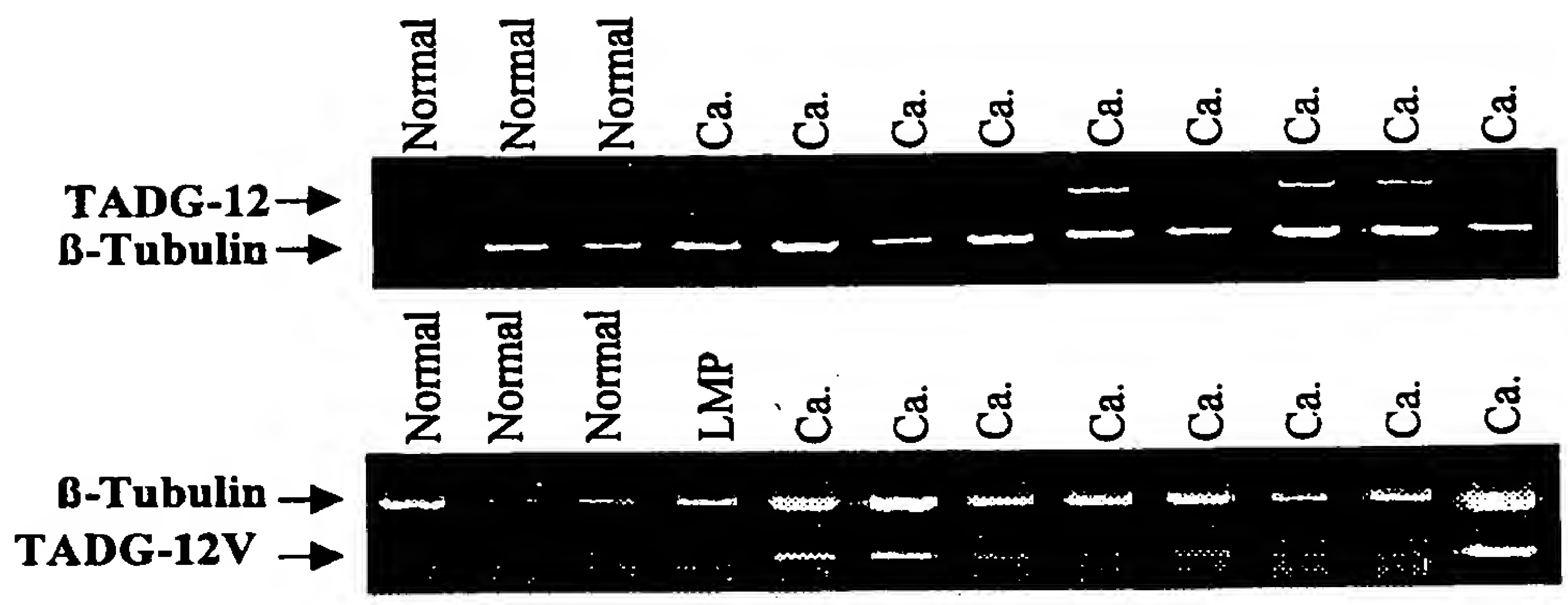

Normal
Normal
Normal
Ca.
Ca.
Ca.
Ca.
Ca.
Ca.
Ca.
Ca.
Ca.
TADG-12
ß-Tubulin
Normal
Normal
Normal
LMP
Ca.
Ca.
Ca.
Ca.
Ca.
Ca.
Ca.
Ca.
ß-Tubulin
TADG-12V

FIG. 6

FIG. 7A

FIG. 7B

FIG. 7C

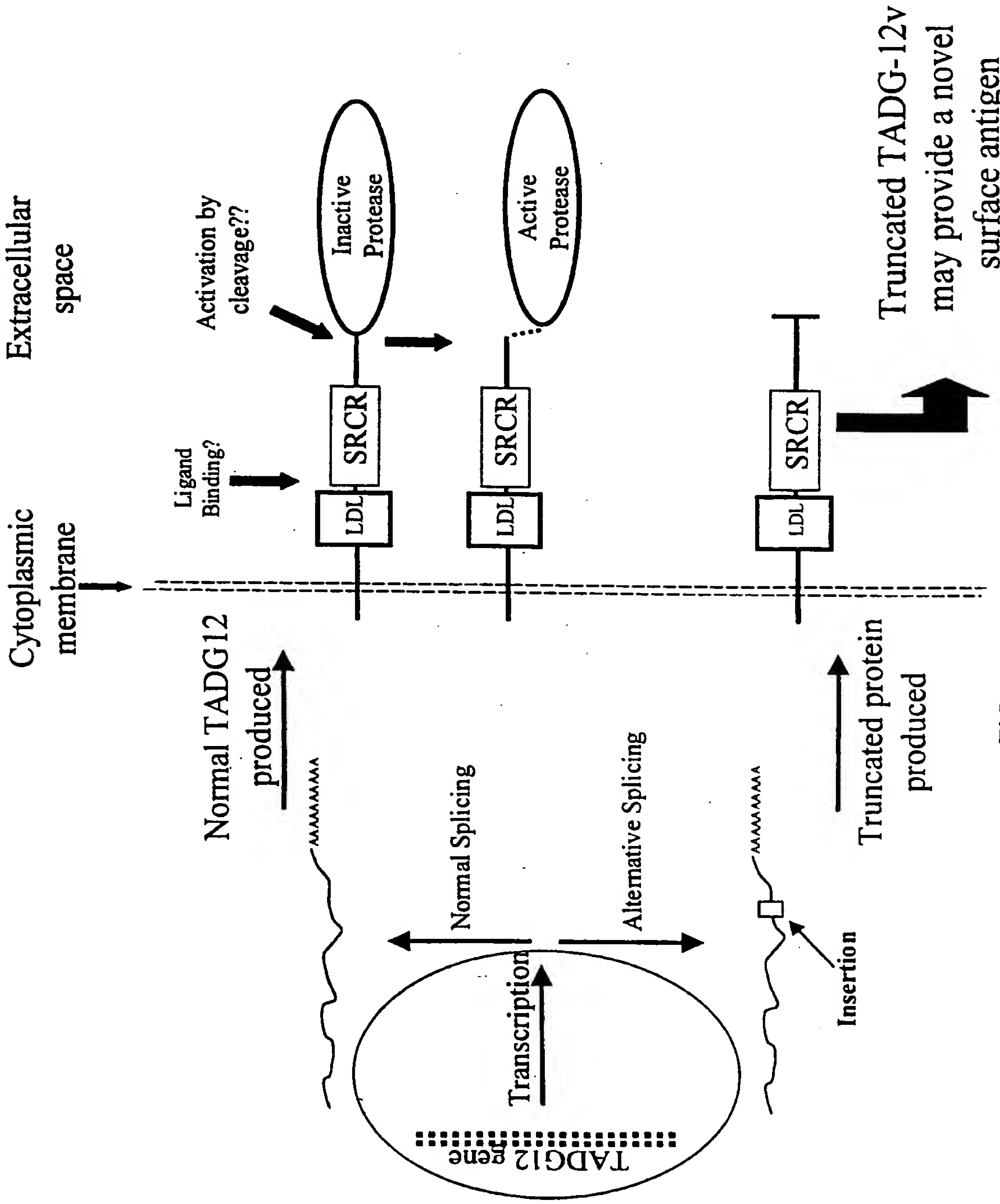

Extracellular space
Cytoplasmic membrane
Ligand Binding?
Activation by cleavage??
Inactive Protease
Active Protease
SRCR
LDL
SRCR
LDL
SRCR
LDL
Truncated TADG-12v may provide a novel surface antigen
Normal TADG12 produced
Truncated protein produced
AAAAAAAA
AAAAAAAA
Normal Splicing
Alternative Splicing
Transcription
TADG12 gene
Insertion

FIG. 8

SEQUENCE LISTING

<110> O'Brien, Timothy J.
Underwood, Lowell J.
<120> Transmembrane Serine Protease Overexpressed in Ovarian Carcinoma and Uses Thereof
<130> D6192PCT
<141> 2000-03-02
<150> 09/261,416
<151> 1999-03-03
<160> 153

<210> 1
<211> 2413
<212> DNA
<213> *Homo sapiens*
<220>
<221> CDS
<223> entire cDNA sequence of TADG-12 gene
<400> 1

```
cgggaaaggg ctgtgtttat gggaagccag taacactgtg gcctactatc   50
tcttccgtgg tgccatctac atttttggga ctcgggaatt atgaggtaga  100
ggtggaggcg gagccggatg tcagaggtcc tgaaatagtc accatggggg  150
aaaatgatcc gcctgctgtt gaagcccccct tctcattccg atcgcttttt  200
ggccttgatg atttgaaaat aagtcctgtt gcaccagatg cagatgctgt  250
tgctgcacag atcctgtcac tgctgccatt tgaagttttt tcccaatcat  300
cgtcattggg gatcattgca ttgatattag cactggccat tggtctgggc  350
atccacttcg actgctcagg gaagtacaga tgtcgctcat cctttaagtg  400
tatcgagctg ataactcgat gtgacggagt ctcggattgc aaagacgggg  450
aggacgagta ccgctgtgtc cgggtgggtg gtcagaatgc cgtgctccag  500
gtgttcacag ctgcttcgtg gaagaccatg tgctccgatg actggaaggg  550
tcactacgca aatgttgcct gtgcccaact gggtttccca agctatgtga  600
gttcagataa cctcagagtg agctcgctgg aggggcagtt ccgggaggag  650
tttgtgtcca tcgatcacct cttgccagat gacaaggtga ctgcattaca  700
ccactcagta tatgtgaggg agggatgtgc ctctggccac gtggttacct  750
tgcagtgcac agcctgtggt catagaaggg gctacagctc acgcatcgtg  800
ggtggaaaca tgtccttgct ctcgcagtgg ccctggcagg ccagccttca  850
gttccagggc taccacctgt gcgggggctc tgtcatcacg cccctgtgga  900
tcatcactgc tgcacactgt gtttatgact tgtacctccc caagtcatgg  950
accatccagg tgggtctagt ttccctgttg gacaatccag ccccatccca 1000
cttggtggag aagattgtct accacagcaa gtacaagcca aagaggctgg 1050
gcaatgacat cgcccttatg aagctggccg ggccactcac gttcaatgaa 1100
atgatccagc ctgtgtgcct gcccaactct gaagagaact tccccgatgg 1150
aaaagtgtgc tggacgtcag gatgggggc cacagaggat ggaggtgacg 1200
cctcccctgt cctgaaccac gcggccgtcc ctttgatttc caacaagatc 1250
tgcaaccaca gggacgtgta cggtggcatc atctcccccct ccatgctctg 1300
cgcgggctac ctgacgggtg gcgtgaacag ctgccagggg gacagcgggg 1350
ggcccctggt gtgtcaagag aggaggctgt ggaagttagt gggagcgacc 1400
agctttggca tcggctgcgc agaggtgaac aagcctgggg tgtacacccg 1450
tgtcacctcc ttcctggact ggatccacga gcagatggag agagacctaa 1500
aaacctgaag aggaagggga caagtagcca cctgagttcc tgaggtgatg 1550
aagacagccc gatcctcccc tggactcccg tgtaggaacc tgcacacgag 1600
cagacaccct tggagctctg agttccggca ccagtagcgg gcccgaaaga 1650
ggcacccttc catctgattc cagcacaacc ttcaagctgc tttttgtttt 1700
ttgtttttttt gaggtggagt ctcgctctgt tgcccaggct ggagtgcagt 1750
```

```
ggcgaaatac cctgctcact gcagcctccg cttccctggt tcaagcgatt 1800
ctcttgcctc agcttcccca gtagctggga ccacaggtgc ccgccaccac 1850
acccaactaa tttttgtatt tttagtagag acagggtttc accatgttgg 1900
ccaggctgct ctcaaacccc tgacctcaaa tgatgtgcct gcttcagcct 1950
cccacagtgc tgggattaca ggcatgggcc accacgccta gcctcacgct 2000
cctttctgat cttcactaag aacaaaagaa gcagcaactt gcaagggcgg 2050
cctttcccac tggtccatct ggttttctct ccagggtctt gcaaaattcc 2100
tgacgagata agcagttatg tgacctcacg tgcaaagcca ccaacagcca 2150
ctcagaaaag acgcaccagc ccagaagtgc agaactgcag tcactgcacg 2200
ttttcatctt tagggaccag aaccaaaccc accctttcta cttccaagac 2250
ttattttcac atgtggggag gttaatctag gaatgactcg tttaaggcct 2300
attttcatga tttctttgta gcatttggtg cttgacgtat tattgtcctt 2350
tgattccaaa taatatgttt ccttccctca aaaaaaaaaa aaaaaaaaaa 2400
aaaaaaaaaa aaa                                         2413
```

```
<210>   2
<211>   454
<212>   PRT
<213>   Homo sapiens
<220>
<223>   complete amino acid sequence of TADG-12
        protein
<400>   2
```

```
Met Gly Glu Asn Asp Pro Pro Ala Val Glu Ala Pro Phe Ser Phe
                5                   10                  15
Arg Ser Leu Phe Gly Leu Asp Asp Leu Lys Ile Ser Pro Val Ala
                20                  25                  30
Pro Asp Ala Asp Ala Val Ala Ala Gln Ile Leu Ser Leu Leu Pro
                35                  40                  45
Phe Glu Val Phe Ser Gln Ser Ser Ser Leu Gly Ile Ile Ala Leu
                50                  55                  60
Ile Leu Ala Leu Ala Ile Gly Leu Gly Ile His Phe Asp Cys Ser
                65                  70                  75
Gly Lys Tyr Arg Cys Arg Ser Ser Phe Lys Cys Ile Glu Leu Ile
                80                  85                  90
Thr Arg Cys Asp Gly Val Ser Asp Cys Lys Asp Gly Glu Asp Glu
                95                  100                 105
Tyr Arg Cys Val Arg Val Gly Gly Gln Asn Ala Val Leu Gln Val
                110                 115                 120
Phe Thr Ala Ala Ser Trp Lys Thr Met Cys Ser Asp Asp Trp Lys
                125                 130                 135
Gly His Tyr Ala Asn Val Ala Cys Ala Gln Leu Gly Phe Pro Ser
                140                 145                 150
Tyr Val Ser Ser Asp Asn Leu Arg Val Ser Ser Leu Glu Gly Gln
                155                 160                 165
Phe Arg Glu Glu Phe Val Ser Ile Asp His Leu Leu Pro Asp Asp
                170                 175                 180
Lys Val Thr Ala Leu His His Ser Val Tyr Val Arg Glu Gly Cys
                185                 190                 195
Ala Ser Gly His Val Val Thr Leu Gln Cys Thr Ala Cys Gly His
                200                 205                 210
Arg Arg Gly Tyr Ser Ser Arg Ile Val Gly Gly Asn Met Ser Leu
                215                 220                 225
Leu Ser Gln Trp Pro Trp Gln Ala Ser Leu Gln Phe Gln Gly Tyr
                230                 235                 240
```

His Leu Cys Gly Gly Ser Val Ile Thr Pro Leu Trp Ile Ile Thr
245 250 255
Ala Ala His Cys Val Tyr Asp Leu Tyr Leu Pro Lys Ser Trp Thr
260 265 270
Ile Gln Val Gly Leu Val Ser Leu Leu Asp Asn Pro Ala Pro Ser
275 280 285
His Leu Val Glu Lys Ile Val Tyr His Ser Lys Tyr Lys Pro Lys
290 295 300
Arg Leu Gly Asn Asp Ile Ala Leu Met Lys Leu Ala Gly Pro Leu
305 310 315
Thr Phe Asn Glu Met Ile Gln Pro Val Cys Leu Pro Asn Ser Glu
320 325 330
Glu Asn Phe Pro Asp Gly Lys Val Cys Trp Thr Ser Gly Trp Gly
335 340 345
Ala Thr Glu Asp Gly Gly Asp Ala Ser Pro Val Leu Asn His Ala
350 355 360
Ala Val Pro Leu Ile Ser Asn Lys Ile Cys Asn His Arg Asp Val
365 370 375
Tyr Gly Gly Ile Ile Ser Pro Ser Met Leu Cys Ala Gly Tyr Leu
380 385 390
Thr Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu
395 400 405
Val Cys Gln Glu Arg Arg Leu Trp Lys Leu Val Gly Ala Thr Ser
410 415 420
Phe Gly Ile Gly Cys Ala Glu Val Asn Lys Pro Gly Val Tyr Thr
425 430 435
Arg Val Thr Ser Phe Leu Asp Trp Ile His Glu Gln Met Glu Arg
440 445 450
Asp Leu Lys Thr

<210> 3
<211> 2544
<212> DNA
<213> *Homo sapiens*
<220>
<221> CDS
<223> entire cDNA sequence of TADG-12 variant gene
<400> 3

cgggaaaggg ctgtgtttat gggaagccag taacactgtg gcctactatc 50
tcttccgtgg tgccatctac atttttggga ctcgggaatt atgaggtaga 100
ggtggaggcg gagccggatg tcagaggtcc tgaaatagtc accatggggg 150
aaaatgatcc gcctgctgtt gaagcccccт tctcattccg atcgcttttt 200
ggccttgatg atttgaaaat aagtcctgtt gcaccagatg cagatgctgt 250
tgctgcacag atcctgtcac tgctgccatt tgaagttttt tcccaatcat 300
cgtcattggg gatcattgca ttgatattag cactggccat tggtctgggc 350
atccacttcg actgctcagg gaagtacaga tgtcgctcat cctttaagtg 400
tatcgagctg ataactcgat gtgacggagt ctcggattgc aaagacgggg 450
aggacgagta ccgctgtgtc cgggtgggtg gtcagaatgc cgtgctccag 500
gtgttcacag ctgcttcgtg gaagaccatg tgctccgatg actggaaggg 550
tcactacgca aatgttgcct gtgcccaact gggtttccca agctatgtaa 600
gttcagataa cctcagagtg agctcgctgg aggggcagtt ccgggaggag 650
tttgtgtcca tcgatcacct cttgccagat gacaaggtga ctgcattaca 700
ccactcagta tatgtgaggg agggatgtgc ctctggccac gtggttacct 750
tgcagtgcac agcctgtggt catagaaggg gctacagctc acgcatcgtg 800

ggtggaaaca tgtccttgct ctcgcagtgg ccctggcagg ccagccttca 850
gttccagggc taccacctgt gcgggggctc tgtcatcacg cccctgtgga 900
tcatcactgc tgcacactgt gtttatgaga ttgtagctcc tagagaaagg 950
gcagacagaa gaggaaggaa gctcctgtgc tggaggaaac ccacaaaaat 1000
gaaaggacct agaccttccc atagctaatt ccagtggacc atgttatggc 1050
agatacaggc ttgtacctcc ccaagtcatg gaccatccag gtgggtctag 1100
tttccctgtt ggacaatcca gccccatccc acttggtgga gaagattgtc 1150
taccacagca agtacaagcc aaagaggctg ggcaatgaca tcgcccttat 1200
gaagctggcc gggccactca cgttcaatga aatgatccag cctgtgtgcc 1250
tgcccaactc tgaagagaac ttccccgatg gaaaagtgtg ctggacgtca 1300
ggatgggggg ccacagagga tggaggtgac gcctcccctg tcctgaacca 1350
cgcggccgtc cctttgattt ccaacaagat ctgcaaccac agggacgtgt 1400
acggtggcat catctccccc tccatgctct gcgcgggcta cctgacgggt 1450
ggcgtggaca gctgccaggg ggacagcggg gggcccctgg tgtgtcaaga 1500
gaggaggctg tggaagttag tgggagcgac cagctttggc atcggctgcg 1550
cagaggtgaa caagcctggg gtgtacaccc gtgtcacctc cttcctggac 1600
tggatccacg agcagatgga gagagaccta aaacctgaa gaggaagggg 1650
acaagtagcc acctgagttc ctgaggtgat gaagacagcc cgatcctccc 1700
ctggactccc gtgtaggaac ctgcacacga gcagacaccc ttggagctct 1750
gagttccggc accagtagcg ggcccgaaag aggcaccctt ccatctgatt 1800
ccagcacaac cttcaagctg ctttttgttt tttgtttttt tgaggtggag 1850
tctcgctctg ttgcccaggc tggagtgcag tggcgaaata ccctgctcac 1900
tgcagcctcc gcttccctgg ttcaagcgat tctcttgcct cagcttcccc 1950
agtagctggg accacaggtg cccgccacca cacccaacta atttttgtat 2000
ttttagtaga gacagggttt caccatgttg gccaggctgc tctcaaaccc 2050
ctgacctcaa atgatgtgcc tgcttcagcc tcccacagtg ctgggattac 2100
aggcatgggc caccacgcct agcctcacgc tcctttctga tcttcactaa 2150
gaacaaaaga agcagcaact tgcaagggcg gcctttccca ctggtccatc 2200
tggttttctc tccagggtct tgcaaaattc ctgacgagat aagcagttat 2250
gtgacctcac gtgcaaagcc accaacagcc actcagaaaa gacgcaccag 2300
cccagaagtg cagaactgca gtcactgcac gttttcatct ttagggacca 2350
gaaccaaacc caccctttct acttccaaga cttattttca catgtgggga 2400
ggttaatcta ggaatgactc gtttaaggcc tattttcatg atttctttgt 2450
agcatttggt gcttgacgta ttattgtcct ttgattccaa ataatatgtt 2500
tccttccctc aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 2544

<210> 4
<211> 294
<212> PRT
<213> *Homo sapiens*
<220>
<223> complete amino acid sequence of TADG-12 variant protein
<400> 4

Met Gly Glu Asn Asp Pro Pro Ala Val Glu Ala Pro Phe Ser Phe
5 10 15
Arg Ser Leu Phe Gly Leu Asp Asp Leu Lys Ile Ser Pro Val Ala
20 25 30
Pro Asp Ala Asp Ala Val Ala Ala Gln Ile Leu Ser Leu Leu Pro
35 40 45
Phe Glu Val Phe Ser Gln Ser Ser Ser Leu Gly Ile Ile Ala Leu
50 55 60
Ile Leu Ala Leu Ala Ile Gly Leu Gly Ile His Phe Asp Cys Ser
65 70 75
Gly Lys Tyr Arg Cys Arg Ser Ser Phe Lys Cys Ile Glu Leu Ile

80 85 90
Thr Arg Cys Asp Gly Val Ser Asp Cys Lys Asp Gly Glu Asp Glu
95 100 105
Tyr Arg Cys Val Arg Val Gly Gly Gln Asn Ala Val Leu Gln Val
110 115 120
Phe Thr Ala Ala Ser Trp Lys Thr Met Cys Ser Asp Asp Trp Lys
125 130 135
Gly His Tyr Ala Asn Val Ala Cys Ala Gln Leu Gly Phe Pro Ser
140 145 150
Tyr Val Ser Ser Asp Asn Leu Arg Val Ser Ser Leu Glu Gly Gln
155 160 165
Phe Arg Glu Glu Phe Val Ser Ile Asp His Leu Leu Pro Asp Asp
170 175 180
Lys Val Thr Ala Leu His His Ser Val Tyr Val Arg Glu Gly Cys
185 190 195
Ala Ser Gly His Val Val Thr Leu Gln Cys Thr Ala Cys Gly His
200 205 210
Arg Arg Gly Tyr Ser Ser Arg Ile Val Gly Gly Asn Met Ser Leu
215 220 225
Leu Ser Gln Trp Pro Trp Gln Ala Ser Leu Gln Phe Gln Gly Tyr
230 235 240
His Leu Cys Gly Gly Ser Val Ile Thr Pro Leu Trp Ile Ile Thr
245 250 255
Ala Ala His Cys Val Tyr Glu Ile Val Ala Pro Arg Glu Arg Ala
260 265 270
Asp Arg Arg Gly Arg Lys Leu Leu Cys Trp Arg Lys Pro Thr Lys
275 280 285
Met Lys Gly Pro Arg Pro Ser His Ser
290

<210> 5
<211> 174
<212> DNA
<213> Artificial sequence
<220>
<223> nucleotide sequence of the subclone containing the 180 bp band from the PCR product for TADG-12
<400> 5

tgggtggtga cggcggcgca ctgtgtttat gacttgtacc tccccaagtc 50
atggaccatc caggtgggtc tagtttccct gttggacaat ccagccccat 100
cccacttggt ggagaagatt gtctaccaca gcaagtacaa gccaaagagg 150
ctgggcaacg acatcgccct ccta 174

<210> 6
<211> 58
<212> PRT
<213> Artificial sequence
<220>
<223> deduced amino acid sequence of the 180 bp band from the PCR product for TADG-12
<400> 6

Trp Val Val Thr Ala Ala His Cys Val Tyr Asp Leu Tyr Leu Pro
5 10 15
Lys Ser Trp Thr Ile Gln Val Gly Leu Val Ser Leu Leu Asp Asn

```
                20                     25                     30
Pro Ala Pro Ser His Leu Val Glu Lys Ile Val Tyr His Ser Lys
                35                     40                     45
Tyr Lys Pro Lys Arg Leu Gly Asn Asp Ile Ala Leu Leu
                50                     55
```

<210> 7
<211> 328
<212> DNA
<213> Artificial sequence
<220>
<223> nucleotide sequence of the subclone containing the 300 bp band from the PCR product for TADG-12 variant, which contains an additional insert of 133 bases
<400> 7

```
gggtggtgac ggcggcgcac tgtgtttatg agattgtagc tcctagagaa    50
agggcagaca gaagaggaag gaagctcctg tgctggagga aacccacaaa   100
aatgaaagga cctagacctt cccatagcta attccagtgg accatgttat   150
ggcagataca ggcttgtacc tccccaagtc atggaccatc caggtgggtc   200
tagtttccct gttggacaat ccagccccat cccacttggt ggagaagatt   250
gtctaccaca gcaagtacaa gccaaagagg ctgggcaacg acatcgccct   300
cctaatcact agtgcggccg cctgcagg                             328
```

<210> 8
<211> 42
<212> PRT
<213> Artificial sequence
<220>
<223> deduced amino acid sequence of the 300 bp band from the PCR product for TADG-12 variant, which is a truncated form of TADG-12
<400> 8

```
Val Val Thr Ala Ala His Cys Val Tyr Glu Ile Val Ala Pro Arg
                5                      10                     15
Glu Arg Ala Asp Arg Arg Gly Arg Lys Leu Leu Cys Trp Arg Lys
                20                     25                     30
Pro Thr Lys Met Lys Gly Pro Arg Pro Ser His Ser
                35                     40
```

<210> 9
<211> 34
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> LDLR-A domain of the complement subunit C8 (Compc8)
<400> 9

```
Cys Glu Gly Phe Val Cys Ala Gln Thr Gly Arg Cys Val Asn Arg
                5                      10                     15
Arg Leu Leu Cys Asn Gly Asp Asn Asp Cys Gly Asp Gln Ser Asp
                20                     25                     30
```

Glu Ala Asn Cys

<210> 10
<211> 34
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> LDLR-A domain of the serine protease matriptase (Matr)
<400> 10

```
Cys Pro Gly Gln Phe Thr Cys Arg Thr Gly Arg Cys Ile Arg Lys
                5                   10                  15
Glu Leu Arg Cys Asp Gly Trp Ala Asp Cys Thr Asp His Ser Asp
                20                  25                  30
Glu Leu Asn Cys
```

<210> 11
<211> 37
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> LDLR-A domain of the glycoprotein GP300 (Gp300-1)
<400> 11

```
Cys Gln Gln Gly Tyr Phe Lys Cys Gln Ser Glu Gly Gln Cys Ile
                5                   10                  15
Pro Ser Ser Trp Val Cys Asp Gln Asp Gln Asp Cys Asp Asp Gly
                20                  25                  30
Ser Asp Glu Arg Gln Asp Cys
                35
```

<210> 12
<211> 35
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> LDLR-A domain of the glycoprotein GP300 (Gp300-2)
<400> 12

```
Cys Ser Ser His Gln Ile Thr Cys Ser Asn Gly Gln Cys Ile Pro
                5                   10                  15
Ser Glu Tyr Arg Cys Asp His Val Arg Asp Cys Pro Asp Gly Ala
                20                  25                  30
Asp Glu Asn Asp Cys
                35
```

<210> 13
<211> 35

<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<222> 74...108
<223> LDLR-A domain of TADG-12
<400> 13

```
Cys Ser Gly Lys Tyr Arg Cys Arg Ser Ser Phe Lys Cys Ile Glu
                5                   10                  15
Leu Ile Thr Arg Cys Asp Gly Val Ser Asp Cys Lys Asp Gly Glu
                20                  25                  30
Asp Glu Tyr Arg Cys
                35
```

<210> 14
<211> 36
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> LDLR-A domain of the serine protease TMPRSS2 Tmprss2
<400> 14

```
Cys Ser Asn Ser Gly Ile Glu Cys Asp Ser Ser Gly Thr Cys Ile
                5                   10                  15
Asn Pro Ser Asn Trp Cys Asp Gly Val Ser His Cys Pro Gly Gly
                20                  25                  30
Glu Asp Glu Asn Arg Cys
                35
```

<210> 15
<211> 101
<212> PRT
<213> *Bos taurus*
<220>
<221> DOMAIN
<223> SRCR domain of bovine enterokinase (BovEntk)
<400> 15

```
Val Arg Leu Val Gly Gly Ser Gly Pro His Glu Gly Arg Val Glu
                5                   10                  15
Ile Phe His Glu Gly Gln Trp Gly Thr Val Cys Asp Asp Arg Trp
                20                  25                  30
Glu Leu Arg Gly Gly Leu Val Val Cys Arg Ser Leu Gly Tyr Lys
                35                  40                  45
Gly Val Gln Ser Val His Lys Arg Ala Tyr Phe Gly Lys Gly Thr
                50                  55                  60
Gly Pro Ile Trp Leu Asn Glu Val Phe Cys Phe Gly Lys Glu Ser
                65                  70                  75
Ser Ile Glu Glu Cys Arg Ile Arg Gln Trp Gly Val Arg Ala Cys
                80                  85                  90
Ser His Asp Glu Asp Ala Gly Val Thr Cys Thr
                95                  100
```

<210> 16
<211> 101
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> SRCR domain of human macrophage scavenger receptor (MacSR)
<400> 16

Val Arg Leu Val Gly Gly Ser Gly Pro His Glu Gly Arg Val Glu
5 10 15
Ile Leu His Ser Gly Gln Trp Gly Thr Ile Cys Asp Asp Arg Trp
20 25 30
Glu Val Arg Val Gly Gln Val Val Cys Arg Ser Leu Gly Tyr Pro
35 40 45
Gly Val Gln Ala Val His Lys Ala Ala His Phe Gly Gln Gly Thr
50 55 60
Gly Pro Ile Trp Leu Asn Glu Val Phe Cys Phe Gly Arg Glu Ser
65 70 75
Ser Ile Glu Glu Cys Lys Ile Arg Gln Trp Gly Thr Arg Ala Cys
80 85 90
Ser His Ser Glu Asp Ala Gly Val Thr Cys Thr
95 100

<210> 17
<211> 98
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<222> 109...206
<223> SRCR domain of TADG-12 (TADG12)
<400> 17

Val Arg Val Gly Gly Gln Asn Ala Val Leu Gln Val Phe Thr Ala
5 10 15
Ala Ser Trp Lys Thr Met Cys Ser Asp Asp Trp Lys Gly His Tyr
20 25 30
Ala Asn Val Ala Cys Ala Gln Leu Gly Phe Pro Ser Tyr Val Ser
35 40 45
Ser Asp Asn Leu Arg Val Ser Ser Leu Glu Gly Gln Phe Arg Glu
50 55 60
Glu Phe Val Ser Ile Asp His Leu Leu Pro Asp Asp Lys Val Thr
65 70 75
Ala Leu His His Ser Val Tyr Val Arg Glu Gly Cys Ala Ser Gly
80 85 90
His Val Val Thr Leu Gln Cys Thr
95

<210> 18
<211> 94
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN

<223> SRCR domain of the serine protease TMPRSS2 (Tmprss2)
<400> 18

Val Arg Leu Tyr Gly Pro Asn Phe Ile Leu Gln Met Tyr Ser Ser
5 10 15
Gln Arg Lys Ser Trp His Pro Val Cys Gln Asp Asp Trp Asn Glu
20 25 30
Asn Tyr Gly Arg Ala Ala Cys Arg Asp Met Gly Tyr Lys Asn Asn
35 40 45
Phe Tyr Ser Ser Gln Gly Ile Val Asp Asp Ser Gly Ser Thr Ser
50 55 60
Phe Met Lys Leu Asn Thr Ser Ala Gly Asn Val Asp Ile Tyr Lys
65 70 75
Lys Leu Tyr His Ser Asp Ala Cys Ser Ser Lys Ala Val Val Ser
80 85 90
Leu Arg Cys Leu

<210> 19
<211> 90
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> SRCR domain of human enterokinase (HumEntk)
<400> 19

Val Arg Phe Phe Asn Gly Thr Thr Asn Asn Asn Gly Leu Val Arg
5 10 15
Phe Arg Ile Gln Ser Ile Trp His Thr Ala Cys Ala Glu Asn Trp
20 25 30
Thr Thr Gln Ile Ser Asn Asp Val Cys Gln Leu Leu Gly Leu Gly
35 40 45
Ser Gly Asn Ser Ser Lys Pro Ile Phe Ser Thr Asp Gly Gly Pro
50 55 60
Phe Val Lys Leu Asn Thr Ala Pro Asp Gly His Leu Ile Leu Thr
65 70 75
Pro Ser Gln Gln Cys Leu Gln Asp Ser Leu Ile Arg Leu Gln Cys
80 85 90

<210> 20
<211> 149
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> protease domain of protease M (ProM)
<400> 20
Leu Trp Val Leu Thr Ala Ala His Cys Lys Lys Pro Asn Leu Gln
5 10 15
Val Phe Leu Gly Lys His Asn Leu Arg Gln Arg Glu Ser Ser Gln
20 25 30
Glu Gln Ser Ser Val Val Arg Ala Val Ile His Pro Asp Tyr Asp
35 40 45
Ala Ala Ser His Asp Gln Asp Ile Met Leu Leu Arg Leu Ala Arg

```
                50                      55                      60
Pro Ala Lys Leu Ser Glu Leu Ile Gln Pro Leu Pro Leu Glu Arg
                65                      70                      75
Asp Cys Ser Ala Asn Thr Thr Ser Cys His Ile Leu Gly Trp Gly
                80                      85                      90
Lys Thr Ala Asp Gly Asp Phe Pro Asp Thr Ile Gln Cys Ala Tyr
                95                      100                     105
Ile His Leu Val Ser Arg Glu Glu Cys Glu His Ala Tyr Pro Gly
                110                     115                     120
Gln Ile Thr Gln Asn Met Leu Cys Ala Gly Asp Glu Lys Tyr Gly
                125                     130                     135
Lys Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
                140                     145
```

```
     <210>     21
     <211>     151
     <212>     PRT
     <213>     Homo sapiens
     <220>
     <221>     DOMAIN
     <223>     protease domain of trypsinogen I (Try1)
     <400>     21
```

```
Gln Trp Val Val Ser Ala Gly His Cys Tyr Lys Ser Arg Ile Gln
                5                       10                      15
Val Arg Leu Gly Glu His Asn Ile Glu Val Leu Glu Gly Asn Glu
                20                      25                      30
Gln Phe Ile Asn Ala Ala Lys Ile Ile Arg His Pro Gln Tyr Asp
                35                      40                      45
Arg Lys Thr Leu Asn Asn Asp Ile Met Leu Ile Lys Leu Ser Ser
                50                      55                      60
Arg Ala Val Ile Asn Ala Arg Val Ser Thr Ile Ser Leu Pro Thr
                65                      70                      75
Ala Pro Pro Ala Thr Gly Thr Lys Cys Leu Ile Ser Gly Trp Gly
                80                      85                      90
Asn Thr Ala Ser Ser Gly Ala Asp Tyr Pro Asp Glu Leu Gln Cys
                95                      100                     105
Leu Asp Ala Pro Val Leu Ser Gln Ala Lys Cys Glu Ala Ser Tyr
                110                     115                     120
Pro Gly Lys Ile Thr Ser Asn Met Phe Cys Val Gly Phe Leu Glu
                125                     130                     135
Gly Gly Lys Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Val Val
                140                     145                     150
Cys
```

```
     <210>     22
     <211>     158
     <212>     PRT
     <213>     Homo sapiens
     <220>
     <221>     DOMAIN
     <223>     protease domain of plasma kallikrein (Kal)
     <400>     22
```

```
Gln Trp Val Leu Thr Ala Ala His Cys Phe Asp Gly Leu Pro Leu
```

```
                5                   10                  15
Gln Asp Val Trp Arg Ile Tyr Ser Gly Ile Leu Asn Leu Ser Asp
                20                  25                  30
Ile Thr Lys Asp Thr Pro Phe Ser Gln Ile Lys Glu Ile Ile Ile
                35                  40                  45
His Gln Asn Tyr Lys Val Ser Glu Gly Asn His Asp Ile Ala Leu
                50                  55                  60
Ile Lys Leu Gln Ala Pro Leu Asn Tyr Thr Glu Phe Gln Lys Pro
                65                  70                  75
Ile Cys Leu Pro Ser Lys Gly Asp Thr Ser Thr Ile Tyr Thr Asn
                80                  85                  90
Cys Trp Val Thr Gly Trp Gly Phe Ser Lys Glu Lys Gly Glu Ile
                95                  100                 105
Gln Asn Ile Leu Gln Lys Val Asn Ile Pro Leu Val Thr Asn Glu
                110                 115                 120
Glu Cys Gln Lys Arg Tyr Gln Asp Tyr Lys Ile Thr Gln Arg Met
                125                 130                 135
Val Cys Ala Gly Tyr Lys Glu Gly Gly Lys Asp Ala Cys Lys Gly
                140                 145                 150
Asp Ser Gly Gly Pro Leu Val Cys
                155

     <210>      23
     <211>      157
     <212>      PRT
     <213>      Homo sapiens
     <220>
     <221>      DOMAIN
     <223>      protease domain of TADG-12 (TADG12)
     <400>      23

Leu Trp Ile Ile Thr Ala Ala His Cys Val Tyr Asp Leu Tyr Leu
                5                   10                  15
Pro Lys Ser Trp Thr Ile Gln Val Gly Leu Val Ser Leu Leu Asp
                20                  25                  30
Asn Pro Ala Pro Ser His Leu Val Glu Lys Ile Val Tyr His Ser
                35                  40                  45
Lys Tyr Lys Pro Lys Arg Leu Gly Asn Asp Ile Ala Leu Met Lys
                50                  55                  60
Leu Ala Gly Pro Leu Thr Phe Asn Glu Met Ile Gln Pro Val Cys
                65                  70                  75
Leu Pro Asn Ser Glu Glu Asn Phe Pro Asp Gly Lys Val Cys Trp
                80                  85                  90
Thr Ser Gly Trp Gly Ala Thr Glu Asp Gly Gly Asp Ala Ser Pro
                95                  100                 105
Val Leu Asn His Ala Ala Val Pro Leu Ile Ser Asn Lys Ile Cys
                110                 115                 120
Asn His Arg Asp Val Tyr Gly Gly Ile Ile Ser Pro Ser Met Leu
                125                 130                 135
Cys Ala Gly Tyr Leu Thr Gly Gly Val Asp Ser Cys Gln Gly Asp
                140                 145                 150
Ser Gly Gly Pro Leu Val Cys
                155

     <210>      24
     <211>      159
```

<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> protease domain of TMPRSS2 (Tmprss2)
<400> 24

Glu Trp Ile Val Thr Ala Ala His Cys Val Glu Lys Pro Leu Asn
5 10 15
Asn Pro Trp His Trp Thr Ala Phe Ala Gly Ile Leu Arg Gln Ser
20 25 30
Phe Met Phe Tyr Gly Ala Gly Tyr Gln Val Gln Lys Val Ile Ser
35 40 45
His Pro Asn Tyr Asp Ser Lys Thr Lys Asn Asn Asp Ile Ala Leu
50 55 60
Met Lys Leu Gln Lys Pro Leu Thr Phe Asn Asp Leu Val Lys Pro
65 70 75
Val Cys Leu Pro Asn Pro Gly Met Met Leu Gln Pro Glu Gln Leu
80 85 90
Cys Trp Ile Ser Gly Trp Gly Ala Thr Glu Glu Lys Gly Lys Thr
95 100 105
Ser Glu Val Leu Asn Ala Ala Lys Val Leu Leu Ile Glu Thr Gln
110 115 120
Arg Cys Asn Ser Arg Tyr Val Tyr Asp Asn Leu Ile Thr Pro Ala
125 130 135
Met Ile Cys Ala Gly Phe Leu Gln Gly Asn Val Asp Ser Cys Gln
140 145 150
Gly Asp Ser Gly Gly Pro Leu Val Thr
155

<210> 25
<211> 164
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> protease domain of Hepsin (Heps)
<400> 25

Asp Trp Val Leu Thr Ala Ala His Cys Phe Pro Glu Arg Asn Arg
5 10 15
Val Leu Ser Arg Trp Arg Val Phe Ala Gly Ala Val Ala Gln Ala
20 25 30
Ser Pro His Gly Leu Gln Leu Gly Val Gln Ala Val Val Tyr His
35 40 45
Gly Gly Tyr Leu Pro Phe Arg Asp Pro Asn Ser Glu Glu Asn Ser
50 55 60
Asn Asp Ile Ala Leu Val His Leu Ser Ser Pro Leu Pro Leu Thr
65 70 75
Glu Tyr Ile Gln Pro Val Cys Leu Pro Ala Ala Gly Gln Ala Leu
80 85 90
Val Asp Gly Lys Ile Cys Thr Val Thr Gly Trp Gly Asn Thr Gln
95 100 105
Tyr Tyr Gly Gln Gln Ala Gly Val Leu Gln Glu Ala Arg Val Pro
110 115 120
Ile Ile Ser Asn Asp Val Cys Asn Gly Ala Asp Phe Tyr Gly Asn

```
                125                     130                     135
Gln Ile Lys Pro Lys Met Phe Cys Ala Gly Tyr Pro Glu Gly Gly
                140                     145                     150
Ile Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Phe Val Cys
                155                     160

     <210>     26
     <211>     23
     <212>     DNA
     <213>     Artificial sequence
     <220>
     <221>     primer_bind
     <222>     6, 9, 12, 15, 18
     <223>     forward redundant primer for the consensus
               sequences of amino acids surrounding the catalytic
               triad for serine proteases, n = inosine
     <400>     26

tgggtngtna cngcngcnca ytg                                          23

     <210>     27
     <211>     20
     <212>     DNA
     <213>     Artificial sequence
     <220>
     <221>     primer_bind
     <222>     3, 6, 9, 12, 15, 18
     <223>     reverse redundant primer for the consensus
               sequences of amino acids surrounding the catalytic
               triad for serine proteases, n = inosine
     <400>     27

arnarngcna tntcnttncc                                              20

     <210>     28
     <211>     20
     <212>     DNA
     <213>     Artificial sequence
     <220>
     <221>     primer_bind
     <223>     forward oligonucleotide primer for TADG-12
               used for quantitative PCR
     <400>     28

gaaacatgtc cttgctctcg                                              20

     <210>     29
     <211>     20
     <212>     DNA
     <213>     Artificial sequence
     <220>
     <221>     primer_bind
     <223>     reverse oligonucleotide primer for TADG-12
               used for quantitative PCR
     <400>     29
```

actaacttcc acagcctcct 20

<210> 30
<211> 20
<212> DNA
<213> Artificial sequence
<220>
<221> primer_bind
<223> forward oligonucleotide primer for TADG-12 variant (TADG-12V) used for quantitative PCR
<400> 30

tccaggtggg tctagtttcc 20

<210> 31
<211> 20
<212> DNA
<213> Artificial sequence
<220>
<221> primer_bind
<223> reverse oligonucleotide primer for TADG-12 variant (TADG-12V) used for quantitative PCR
<400> 31

ctctttggct tgtacttgct 20

<210> 32
<211> 20
<212> DNA
<213> Artificial sequence
<220>
<221> primer_bind
<223> forward oligonucleotide primer for β-tubulin used as an internal control for quantitative PCR
<400> 32

cgcatcaacg tgtactacaa 20

<210> 33
<211> 20
<212> DNA
<213> Artificial sequence
<220>
<221> primer_bind
<223> reverse oligonucleotide primer for β-tubulin used as an internal control for quantitative PCR
<400> 33

tacgagctgg tggactgaga 20

<210> 34
<211> 12
<212> PRT
<213> Artificial sequence
<220>
<223> a poly-lysine linked multiple antigen peptide

derived from the TADG-12 carboxy-terminal protein sequence, present in full length TADG-12, but not in TADG-12V
<400> 34

Trp Ile His Glu Gln Met Glu Arg Asp Leu Lys Thr
5 10

<210> 35
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 40...48
<223> TADG-12 peptide
<400> 35

Ile Leu Ser Leu Leu Pro Phe Glu Val
5

<210> 36
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 144...152
<223> TADG-12 peptide
<400> 36

Ala Gln Leu Gly Phe Pro Ser Tyr Val
5

<210> 37
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 225...233
<223> TADG-12 peptide
<400> 37

Leu Leu Ser Gln Trp Pro Trp Gln Ala
5

<210> 38
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 252...260
<223> TADG-12 peptide
<400> 38

Trp Ile Ile Thr Ala Ala His Cys Val
5

<210> 39
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 356...364
<223> TADG-12 peptide
<400> 39

Val Leu Asn His Ala Ala Val Pro Leu
5

<210> 40
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 176...184
<223> TADG-12 peptide
<400> 40

Leu Leu Pro Asp Asp Lys Val Thr Ala
5

<210> 41
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 13...21
<223> TADG-12 peptide
<400> 41

Phe Ser Phe Arg Ser Leu Phe Gly Leu
5

<210> 42
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 151...159
<223> TADG-12 peptide
<400> 42

Tyr Val Ser Ser Asp Asn Leu Arg Val
5

<210> 43
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 436...444
<223> TADG-12 peptide
<400> 43

Arg Val Thr Ser Phe Leu Asp Trp Ile
5

<210> 44
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 234...242
<223> TADG-12 peptide
<400> 44

Ser Leu Gln Phe Gln Gly Tyr His Leu
5

<210> 45
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 181...189
<223> TADG-12 peptide
<400> 45

Lys Val Thr Ala Leu His His Ser Val
5

<210> 46
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 183...191
<223> TADG-12 peptide
<400> 46

Thr Ala Leu His His Ser Val Tyr Val
5

<210> 47
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 411...419
<223> TADG-12 peptide
<400> 47

Arg Leu Trp Lys Leu Val Gly Ala Thr
5

<210> 48
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<222> 60...68
<223> TADG-12 peptide
<400> 48

Leu Ile Leu Ala Leu Ala Ile Gly Leu
5

<210> 49
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 227...235
<223> TADG-12 peptide
<400> 49

Ser Gln Trp Pro Trp Gln Ala Ser Leu
5

<210> 50
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 301...309
<223> TADG-12 peptide
<400> 50

Arg Leu Gly Asn Asp Ile Ala Leu Met
5

<210> 51
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 307...315
<223> TADG-12 peptide
<400> 51

Ala Leu Met Lys Leu Ala Gly Pro Leu
5

<210> 52
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 262...270
<223> TADG-12 peptide
<400> 52

Asp Leu Tyr Leu Pro Lys Ser Trp Thr
5

<210> 53
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 416...424
<223> TADG-12 peptide
<400> 53

Leu Val Gly Ala Thr Ser Phe Gly Ile
5

<210> 54
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 54...62
<223> TADG-12 peptide
<400> 54

Ser Leu Gly Ile Ile Ala Leu Ile Leu
5

<210> 55
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 218...226
<223> TADG-12 peptide
<400> 55

Ile Val Gly Gly Asn Met Ser Leu Leu
5

<210> 56
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 35...43
<223> TADG-12 peptide
<400> 56

Ala Val Ala Ala Gln Ile Leu Ser Leu
5

<210> 57
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 271...279
<223> TADG-12 peptide
<400> 57

Ile Gln Val Gly Leu Val Ser Leu Leu
5

<210> 58
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 397...405
<223> TADG-12 peptide
<400> 58

Cys Gln Gly Asp Ser Gly Gly Pro Leu
5

<210> 59
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 270...278
<223> TADG-12 peptide
<400> 59

Thr Ile Gln Val Gly Leu Val Ser Leu
5

<210> 60
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 56...64
<223> TADG-12 peptide
<400> 60

Gly Ile Ile Ala Leu Ile Leu Ala Leu
5

<210> 61
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 110...118
<223> TADG-12 peptide
<400> 61

Arg Val Gly Gly Gln Asn Ala Val Leu
5

<210> 62
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<222> 217...225
<223> TADG-12 peptide
<400> 62

Arg Ile Val Gly Gly Asn Met Ser Leu
5

<210> 63
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 130...138
<223> TADG-12 peptide
<400> 63

Cys Ser Asp Asp Trp Lys Gly His Tyr
5

<210> 64
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 8...16
<223> TADG-12 peptide
<400> 64

Ala Val Glu Ala Pro Phe Ser Phe Arg
5

<210> 65
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 328...336
<223> TADG-12 peptide
<400> 65

Asn Ser Glu Glu Asn Phe Pro Asp Gly
5

<210> 66
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 3...11
<223> TADG-12 peptide
<400> 66

Glu Asn Asp Pro Pro Ala Val Glu Ala
5

<210> 67
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 98...106
<223> TADG-12 peptide
<400> 67

Asp Cys Lys Asp Gly Glu Asp Glu Tyr
5

<210> 68
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 346...354
<223> TADG-12 peptide
<400> 68

Ala Thr Glu Asp Gly Gly Asp Ala Ser
5

<210> 69
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 360...368
<223> TADG-12 peptide
<400> 69

Ala Ala Val Pro Leu Ile Ser Asn Lys
5

<210> 70
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 153...161
<223> TADG-12 peptide
<400> 70

Ser Ser Asp Asn Leu Arg Val Ser Ser
5

<210> 71
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 182...190
<223> TADG-12 peptide
<400> 71

Val Thr Ala Leu His His Ser Val Tyr
5

<210> 72
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 143...151
<223> TADG-12 peptide
<400> 72

Cys Ala Gln Leu Gly Phe Pro Ser Tyr
5

<210> 73
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 259...267
<223> TADG-12 peptide
<400> 73

Cys Val Tyr Asp Leu Tyr Leu Pro Lys
5

<210> 74
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 369...377
<223> TADG-12 peptide
<400> 74

Ile Cys Asn His Arg Asp Val Tyr Gly
5

<210> 75
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 278...286
<223> TADG-12 peptide
<400> 75

Leu Leu Asp Asn Pro Ala Pro Ser His
5

<210> 76
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<222> 426...434
<223> TADG-12 peptide
<400> 76

Cys Ala Glu Val Asn Lys Pro Gly Val
5

<210> 77
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 32...40
<223> TADG-12 peptide
<400> 77

Asp Ala Asp Ala Val Ala Ala Gln Ile
5

<210> 78
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 406...414
<223> TADG-12 peptide
<400> 78

Val Cys Gln Glu Arg Arg Leu Trp Lys
5

<210> 79
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 329...337
<223> TADG-12 peptide
<400> 79

Ser Glu Glu Asn Phe Pro Asp Gly Lys
5

<210> 80
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 303...311
<223> TADG-12 peptide
<400> 80

Gly Asn Asp Ile Ala Leu Met Lys Leu
5

<210> 81
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 127...135
<223> TADG-12 peptide
<400> 81

Lys Thr Met Cys Ser Asp Asp Trp Lys
5

<210> 82
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 440...448
<223> TADG-12 peptide
<400> 82

Phe Leu Asp Trp Ile His Glu Gln Met
5

<210> 83
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 433...441
<223> TADG-12 peptide
<400> 83

Val Tyr Thr Arg Val Thr Ser Phe Leu
5

<210> 84
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 263...271
<223> TADG-12 peptide
<400> 84

Leu Tyr Leu Pro Lys Ser Trp Thr Ile
5

<210> 85
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 169...177
<223> TADG-12 peptide
<400> 85

Glu Phe Val Ser Ile Asp His Leu Leu
5

<210> 86
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 296...304
<223> TADG-12 peptide
<400> 86

Lys Tyr Lys Pro Lys Arg Leu Gly Asn
5

<210> 87
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 16...24
<223> TADG-12 peptide
<400> 87

Arg Ser Leu Phe Gly Leu Asp Asp Leu
5

<210> 88
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 267...275
<223> TADG-12 peptide
<400> 88

Lys Ser Trp Thr Ile Gln Val Gly Leu
5

<210> 89
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 81...89
<223> TADG-12 peptide
<400> 89

Arg Ser Ser Phe Lys Cys Ile Glu Leu
5

<210> 90
<211> 9
<212> PRT

<213> *Homo sapiens*
<220>
<222> 375...383
<223> TADG-12 peptide
<400> 90

Val Tyr Gly Gly Ile Ile Ser Pro Ser
5

<210> 91
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 110...118
<223> TADG-12 peptide
<400> 91

Arg Val Gly Gly Gln Asn Ala Val Leu
5

<210> 92
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 189...197
<223> TADG-12 peptide
<400> 92

Val Tyr Val Arg Glu Gly Cys Ala Ser
5

<210> 93
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 165...173
<223> TADG-12 peptide
<400> 93

Gln Phe Arg Glu Glu Phe Val Ser Ile
5

<210> 94
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 10...18
<223> TADG-12 peptide
<400> 94

Glu Ala Pro Phe Ser Phe Arg Ser Leu
5

<210> 95
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 407...415
<223> TADG-12 peptide
<400> 95

Cys Gln Glu Arg Arg Leu Trp Lys Leu
5

<210> 96
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 381...389
<223> TADG-12 peptide
<400> 96

Ser Pro Ser Met Leu Cys Ala Gly Tyr
5

<210> 97
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 375...383
<223> TADG-12 peptide
<400> 97

Val Tyr Gly Gly Ile Ile Ser Pro Ser
5

<210> 98
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 381...389
<223> TADG-12 peptide
<400> 98

Ser Pro Ser Met Leu Cys Ala Gly Tyr
5

<210> 99
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 362...370
<223> TADG-12 peptide

<400> 99

Val Pro Leu Ile Ser Asn Lys Ile Cys
5

<210> 100
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 373...381
<223> TADG-12 peptide
<400> 100

Arg Asp Val Tyr Gly Gly Ile Ile Ser
5

<210> 101
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 283...291
<223> TADG-12 peptide
<400> 101

Ala Pro Ser His Leu Val Glu Lys Ile
5

<210> 102
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 177...185
<223> TADG-12 peptide
<400> 102

Leu Pro Asp Asp Lys Val Thr Ala Leu
5

<210> 103
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 47...55
<223> TADG-12 peptide
<400> 103

Glu Val Phe Ser Gln Ser Ser Ser Leu
5

<210> 104
<211> 9
<212> PRT

<213> *Homo sapiens*
<220>
<222> 36...44
<223> TADG-12 peptide
<400> 104

Val Ala Ala Gln Ile Leu Ser Leu Leu
5

<210> 105
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 255...263
<223> TADG-12 peptide
<400> 105

Thr Ala Ala His Cys Val Tyr Asp Leu
5

<210> 106
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 138...146
<223> TADG-12 peptide
<400> 106

Tyr Ala Asn Val Ala Cys Ala Gln Leu
5

<210> 107
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 195...203
<223> TADG-12 peptide
<400> 107

Cys Ala Ser Gly His Val Val Thr Leu
5

<210> 108
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 215...223
<223> TADG-12 peptide
<400> 108

Ser Ser Arg Ile Val Gly Gly Asn Met
5

<210> 109
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 298...306
<223> TADG-12 peptide
<400> 109

Lys Pro Lys Arg Leu Gly Asn Asp Ile
5

<210> 110
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 313...321
<223> TADG-12 peptide
<400> 110

Gly Pro Leu Thr Phe Asn Glu Met Ile
5

<210> 111
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 108...116
<223> TADG-12 peptide
<400> 111

Cys Val Arg Val Gly Gly Gln Asn Ala
5

<210> 112
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 294...302
<223> TADG-12 peptide
<400> 112

His Ser Lys Tyr Lys Pro Lys Arg Leu
5

<210> 113
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 265...273
<223> TADG-12 peptide

<400> 113

Leu Pro Lys Ser Trp Thr Ile Gln Val
5

<210> 114
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 88...96
<223> TADG-12 peptide
<400> 114

Glu Leu Ile Thr Arg Cys Asp Gly Val
5

<210> 115
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 79...87
<223> TADG-12 peptide
<400> 115

Arg Cys Arg Ser Ser Phe Lys Cys Ile
5

<210> 116
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 255...263
<223> TADG-12 peptide
<400> 116

Thr Ala Ala His Cys Val Tyr Asp Leu
5

<210> 117
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 207...215
<223> TADG-12 peptide
<400> 117

Ala Cys Gly His Arg Arg Gly Tyr Ser
5

<210> 118
<211> 9
<212> PRT

<213> *Homo sapiens*
<220>
<222> 154...162
<223> TADG-12 peptide
<400> 118

Ser Asp Asn Leu Arg Val Ser Ser Leu
5

<210> 119
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 300...308
<223> TADG-12 peptide
<400> 119

Lys Arg Leu Gly Asn Asp Ile Ala Leu
5

<210> 120
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 435...443
<223> TADG-12 peptide
<400> 120

Thr Arg Val Thr Ser Phe Leu Asp Trp
5

<210> 121
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 376...384
<223> TADG-12 peptide
<400> 121

Tyr Gly Gly Ile Ile Ser Pro Ser Met
5

<210> 122
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 410...418
<223> TADG-12 peptide
<400> 122

Arg Arg Leu Trp Lys Leu Val Gly Ala
5

<210> 123
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 210...218
<223> TADG-12 peptide
<400> 123

His Arg Arg Gly Tyr Ser Ser Arg Ile
5

<210> 124
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 109...117
<223> TADG-12 peptide
<400> 124

Val Arg Val Gly Gly Gln Asn Ala Val
5

<210> 125
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 191...199
<223> TADG-12 peptide
<400> 125

Val Arg Glu Gly Cys Ala Ser Gly His
5

<210> 126
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 78...86
<223> TADG-12 peptide
<400> 126

Tyr Arg Cys Arg Ser Ser Phe Lys Cys
5

<210> 127
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 113...121
<223> TADG-12 peptide

<400> 127

Gly Gln Asn Ala Val Leu Gln Val Phe
5

<210> 128
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 91...99
<223> TADG-12 peptide
<400> 128

Thr Arg Cys Asp Gly Val Ser Asp Cys
5

<210> 129
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 38...46
<223> TADG-12 peptide
<400> 129

Ala Gln Ile Leu Ser Leu Leu Pro Phe
5

<210> 130
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 211...219
<223> TADG-12 peptide
<400> 130

Arg Arg Gly Tyr Ser Ser Arg Ile Val
5

<210> 131
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 216...224
<223> TADG-12 peptide
<400> 131

Ser Arg Ile Val Gly Gly Asn Met Ser
5

<210> 132
<211> 9
<212> PRT

<213> *Homo sapiens*
<220>
<222> 118...126
<223> TADG-12 peptide
<400> 132

Leu Gln Val Phe Thr Ala Ala Ser Trp
5

<210> 133
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 370...378
<223> TADG-12 peptide
<400> 133

Cys Asn His Arg Asp Val Tyr Gly Gly
5

<210> 134
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 393...401
<223> TADG-12 peptide
<400> 134

Gly Val Asp Ser Cys Gln Gly Asp Ser
5

<210> 135
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 235...243
<223> TADG-12 peptide
<400> 135

Leu Gln Phe Gln Gly Tyr His Leu Cys
5

<210> 136
<211> 9
<212> PRT
<213> *Homo sapiens*
<220>
<222> 427...435
<223> TADG-12 peptide
<400> 136

Ala Glu Val Asn Lys Pro Gly Val Tyr
5